STUDIES ON POSSIBLE TISSUE SPECIFICITY OF ENZYMES WITH SPECIAL REFERENCE TO CLINICALLY IMPORTANT SERUM ENZYMES

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DEPARTMENT OF BIOCHEMISTRY
JAWAHARLAL NEHRU MEDICAL COLLEGE
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ALIGARH

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TO MY

PARENTS AND SISTER

DEPARTMENT OF BIOCHEMISTRY
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CERTIFICATE

This is to certify that the work and techniques mentioned in this thesis entitled **"STUDIES ON POSSIBLE TISSUE SPECIFICITY OF ENZYMES WITH SPECIAL REFERENCE TO CLINICALLY IMPORTANT SERUM ENZYMES"** by Dr. Partha Sarkar for the degree of M.D. (Biochemistry) has been undertaken by the candidate himself under our guidance and direct supervision, in the Department of Biochemistry, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh.

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LIST OF ABBREVIATIONS USED

ACP	:	Acid phosphatase
AMP, ADP, ATP	:	Adenosine 5'-mono-di-triphosphate
ALP	:	Alkaline phosphatase
ALT	:	Alanine aminotransferase
AST	:	Aspartate aminotransferase
CPK	:	Creatine phosphokinase
DNA	:	Deoxyribonucleic acid
DNPH	:	Dinitrophenyl hydrazine.
E.C.(followed by numbers)	:	Enzyme commission.
EDTA	:	Ethylene diamine tetra-acetic acid.
FDP	:	Fructose 1,6-diphosphate
F-1-P	:	Frustose-1-phosphate
$\Delta G^{\sim}_{o}$	:	Free energy change
HBD	:	Hydroxy butyrate dehydrogenase.
I.U.	:	International Unit
K.A. (unit)	:	King-Armstrong.
LDH	:	Lactate dehydrogenase
NAD^{+}, NADH	:	Nicotinamide adenine dinucleotide and its' reduced form.
O.D.	:	*Optical Density*
PAP	:	Prostatic acid phosphatase.
S.U.	:	Somogyi Unit.

INTRODUCTION

I. INTRODUCTION

An enzyme is a biocatalyst that catalyses a thermodynamically feasible reaction so that the rate of the reaction is comptaible with the biochemical process essential for the maintenance of a cell.

The striking characteristics of all enzymes are their catalytic power and specificity. The original model of a catalytic site, proposed by Emil Fischer is analogus to lock and key arrangement or rigid template model. The 'induced fit' model of Koshland is the more accepted hypothesis. An essential feature of the latter model is the flexibility of the catalytic site unlike the Fischer model where the catalytic site is supposed to be preshaped to fit the substrate.

In the induced fit model, the substrate induces a conformational change in the enzyme. This aligns amino acid residues or other groups on the enzyme in the correct special orientation for the substrate binding and catalysis or both. Simultaneously other amino acid residues may become buried in the interior of the molecule.

A. General Properties of Enzymes:

Enzymes unlike inorganic catalyst are highly specific

and efficient. The enzyme molecule is able to specifically select it s substrate through interaction at the active centre. Only a fraction of total amino acid residues in an enzyme molecule constitutes active centre or active site. The geometry of active site is maintained by the rest of the amino acid sequence of the enzyme. The active centre usually takes the shape of a cleft where the dielectric constant of the medium is low. A low dielectric oonstant favours non-covalent interaction of the enzyme with the substrate.

An enzyme usually exhibits group specificity i.e. acts only on particular chemical groupings. Thus a series of aldohexoses may be phosphorylated by a kinase and ATP. If the enzyme attacks only one substrate it is called absolute group specificity. It may have a relative group specificity, if it attacks a homologous series of aldohexoses. Another important aspect of enzyme specificity is the enzyme's stereospecificity towards the substrate. With the exception of epimerases, which interconvert optical isomers, enzymes generally show absolute optical specificity for at least a portion of a substrate molecule. Many substrates apparently form three bonds with enzymes. This 'three point attachment' gives asymmetry on an otherwise symmetric molecule.

Enzymes do not alter reaction equilibria as it accelerates the forward reaction by the same factor. In other

words, they accelerate the attainment of equilibria but do not shift their positions.

Enzymes decrease the activation energies (ΔG) of reaction, catalysed by them and thus accelerate the reactions by increasing the fraction of molecules having free energy equal to or greater than ΔG. ($\Delta G = G_{\text{transition state}} - G_{\text{substrate}}$).

The rate of reaction catalysed by an enzyme is markedly influenced by concentration of enzyme and substrate, pH of the media and temperature at which the reaction is carried out. Other variables which affect enzyme catalysed reaction include activators and inhibitors.

Most of the enzyme catalysed reactions follow Michaelis-Menten kinetics. Michaelis-Menten equation is based on steady state hypothesis. Mathematically the Michaelis-Menten equation can be expressed as:

$$v = \frac{V_{max}\,(S)}{K_m + (S)} \qquad \ldots\ (1)$$

Where V is observed velocity at a substrate concentration S; K_m is Michaelis Menten constant and V_{max} is maximum attainable velocity obtained at saturation concentration of the substrate. The steady state hypothesis, simply stated,

implies that the concentration of ES complex remains the same regardless of time. The complex does not accumulate during the reaction because the rate of it s formation is equal to the rate of dissociation.

When v is plotted against S, a rectangular hyperbolic curve is obtained. The value of V_{max} can be computed from extrapolation of the saturation curve to zero substrate concentration. According to equation (1) K_m is equal to substrate concentration where the observed velocity is half of the maximum attainable velocity. The constant, K_m, measures the affintiy of the enzyme for the substrate. The constants K_m and V_{max} together define efficiency of the enzyme and can be determined graphically by plotting 1/V vs. 1/(S) as suggested by Lineweaver and Burk (1)

Among the external variables which are known to influence enzyme activity are pH and temperature. pH influences the state of ionisation of ionizable groups in enzyme/substrate molecule. At extreme pH values, the molecules may undergo gross conformational change which will drastically disrupt the native enzyme conformation and thereby abolish it s enzymatic activity. The pH activity profile is often bell shaped with 'acid' and 'alkaline' arms and a point/region where enzyme shows maximum activity. This pH is called pH optimum.

Temperature influences enzyme catalysed reaction by (i) increasing number of effective collisions between substrate molecules and (ii) by denaturing the enzyme. The first factor enhances the rate of reaction whereas the second will have the opposite effect. At low temperature, velocity increases with increase in temperature because of the preponderance of first factor till a temperature is reached beyond which further heat will decrease the enzyme activity due to the fact that the second factor offsets the first one. The temperature at which maximum enzyme activity is obtained is called optimum temperature.

B. Regulation of Enzyme Activity: The regulation of activity of enzyme in body tissue is viatally important for overall biological control and especially the rate of metabolism. The activity can be regulated either by controlling the cellular level of enzymes or by influencing the catalytic efficiency of enzyme. The latter represents a finer control.

The level of enzyme in body tissue is determined by the rate at which the enzyme is synthesized as well as by the rate of degradation. The biosynthesis of enzyme is primarily controlled at the level of DNA. The gene expression can be induced, repressed or derepressed. Degradation of enzyme has attracted considerable attention in recent years. Structural features of the enzyme which are prone to proteolytic attack have been studied (1a). Although a great deal

has been learned regarding the stability of enzyme in vivo, much remains to be done for formulating a general mechanism of protein degradation. Regardless of the mechanism of protein degradation, the process seems to be regulated by the level of proteinases and proteinase inhibitors.

The catalytic activity of enzyme can also be regulated either by covalent modification or by the help of effectors or modulators. The covalent modification may include conversion of zymogen to enzyme and chemical modification of specific amino acid residues on enzyme molecules. In allosteric regulation, low molecular weight substances including substrates can influence the enzyme activity by non-covalent interaction with the enzyme. The binding of effectors or modulators may shift the equilibrium $T \longleftrightarrow R$, where T and R represents respectively, tense and relaxed conformation of enzyme. In the relaxed conformation, the enzyme possesses marked affinity for substrate whereas in tense conformation, it has negligible affinity for the substrate.

C. Classification of Enzymes:

The enzymes are classified rationally on the basis of reaction types and reaction mechanisms. In this I.U.B. system of classification, enzymes are divided into six major classes, each with 4-13 subclasses.

TABLE 1: CLASSIFICATION OF ENZYMES.

Main class and subclasses	Example
1. **Oxidoreductases:**	
1.1. Acting on $>$CHOH gr. of donors	
1.1.1. with NAD or NADP as acceptor	Alcohol dehydrogenase, IDH glucose oxidase.
1.1.3. with O_2 as acceptor	
1.2. Acting on CHO or CO gr. of donors	
1.2.1. with NAD or NADP as acceptor.	Glyceroldehyde-3-phosphate dehydrogenase
1.2.3. with O_2 as acceptor	Xanthine oxidase.
2. **Transferases:**	
2.1. Transferring C_1 groups	
2.1.1. Methyl transferases	Guanidino acetate methyl transferase.
2.1.3. Carboxyl and carbamoyl transferase	Ornithine carbamoyl transferase.
2.3. Acyl transferases	Choline acetyl transferase.
2.6. Transfering N-containing groups	
2.6.1. Aminotransferases	Transaminases.
3. **Hydrolases:**	
3.1. Cleaving ester linkages	
3.1.1. Carboxylic ester hydrolases	Esterases, lipases
3.1.3. Phosphoric monoester hydrolases	Phosphatases
3.2. Cleaving glycosides	
3.2.1. Glycosidase	Amylase, B glucosidase
3.2.2. N-glycosidase	Nucleosidases

TABLE 1: CONTINUED...

Main class and subclasses	Examples
3.4. Cleaving peptide linkages	
3.4.1. α-amino peptide amino acid hydrolases	Leucine aminopeptidase
3.4.4. Peptidopeptide hydrolases	Pepsin, Trypsin, chymotrypsin.
4. Lyases:	
4.1. C-C lyases	
4.1.1. Carboxylases	Pyruvate decarboxylase
4.1.2. Aldehyde lyases	Aldolases
4.2. C-O lyases	
4.2.1. Hydrolyases	Fumerate hydrolase
5. Isomerases:	
5.1. Racemases and epimerases	
5.1.3. Acting on carbohydrate	Ribulose-5-phosphate epimerase
5.2. Cis-trans isomerases	Maleyl acetoacetate isomerase
5.3. Intramolecular oxidoreductase	Glucose phosphate isomerase.
5.4. Intramolecular transferases	Methylmalonyl COA mutase
6. Ligases:	
6.1. Forming C-O bands	
6.1.1. Amino acid RNA ligases	Amino acid activating enzyme.
6.3. Forming C-N bands	
6.3.1. Acid ammonia ligases	Glutamine synthetase
6.4. Forming C-C bands	
6.4.1. Carboxylases	Acetyl COA carboxylase.

Each enzyme has a systemic code number (EC) consisting of four digits. The first digit indicates class and characterizes the reaction type of the six main classes of enzymes. The second digit represents the subclass, third digit indicates subsubclass and the fourth digit is for the particular enzyme named. The first three digits give a clear indication of the nature of the enzyme. Some examples are summarized in Table-1 (1).

D. Tissue Specificity and Clinical Significance of Enzymes:

The clinical significance of an enzyme directly correlates with it s tissue specificity. Tissue distribution or specificity of an enzyme (or it s isozyme forms) primarily depends on the metabolic demand of the enzyme by the tissue. For example the relative tissue distribution of LDH, and LDH_5 depends upon the oxygen tension of the tissue, and so LDH_1 is predominantly present in oxygen rich heart whereas LDH_5 is the dominant form in oxygen poor muscle. Different forms of malate dehydrogenase recognised in mitochondria and cytosol play different metabolic roles. During differentiation and development of adult tissues from their embryonic one, the isozyme pattern in tissues changes again because of the changing metabolic needs of the tissue. Thus BB isozyme of CPK is predominent in all embryonic tissues; but the expression of the gene for this isozyme is severely restricted in adult.

The expression of gene for this isozyme in brain is, however, expressed at a normal rate even in adult. In myocardium, MM and MB isozymes gradually replace BB as the M gene is expressed in association with myofibrillar contractile elements. Likewise in adult skeletal muscle, the isozymes MM predominates due to increased expression of Mgene. The genes which are expressed in foetal stage are often expressed in malignancy. Thus aldolase A which is a foetal form for liver, reappears in a hepatoma replacing aldolase B, normally present in adult liver tissue.

Enzymes, specific for a particular tissue, varies in concentration in the healthy and diseased states of that tissue. Intracellular enzyme concentration estimation reflects the actual extent of injury but on routine basis such assays are not done. Alteration of serum enzyme level is exploited as a parameter of severity of cellular assault as there is release of enzymes in the plasma following cell damage or death. Other body fluids like C.S.F., RBC or WBC lysate or urine may also be used as the source of estimation.

Inspite of the fact that the attempt of using one individual enzyme as a simple marker suggestive of any disease of a specific tissue isyet to be fulfilled, the epoch-making discovery of estimation of serum lipase in the diagnosis of pancreatic diseases in early 1900 A.D. and the importance of

AST in myocardial infarction observed by LaDue, Wroblewski and Karmin (2) in 1954 have paved the way to exploit the serum enzyme level as diagnostic and prognostic indicator as well, in clinical biochemistry. The diseases of liver, skeletal muscle, bone and other tissues can be diagnosed by alteration of some serum enzymes or more precisely the isozyme fraction of an enzyme to sharpen the diagnostic indicator.

The study of several enzyme or isozyme that changes over a period of hours or days is the best guide in this respect.

Duration of increased enzyme activity is frequently of diagnostic importance. As in case of acute myocardial infarction, the changes in enzyme activity with time varies in a following way:

	CPK	AST	LDH	HBD
Rise begins	4 hours	12-18 hrs	13-18 hrs	12-18 hrs.
Peak activity	36 hours	48 hours	72 hours	72 hours
Return to normal	4 days	5 days	7-10 days	10-14 days

In reversibly inflammatory processes, characterised by increased membrane permeability, cell sap enzymes are more likely to be released into the circulation than are mito-

chondrial enzymes. In necrotic condition, the reverse occurs.

The interpretation of serum enzyme patterns can be difficult if a disease leads to enzyme release from several organs. As in patient with myocardial infarction complicated by heart failure, AST rises first, followed by ALT rise due to hepatic congestion with heart failure.

The difference in the clearance rates of enzymes are widely used diagnostically. The half life of enzymes is important to determine the timing for enzyme estimation.

The base line level of most enzymes and isozymes reflect the normal cell leakage and cellular turn over. Increase in levels of enzymes and isozymes in excess of these reference ranges are associated with a variety of pathological changes and are the basis of clinical utility of enzyme and isozyme estimation.

The normal level of enzyme depends on the assay procedure also and so the method of assay should be mentioned with the normal level of any enzyme.

E. Some Clinically Important Enzymes:

A few clinically important enzymes with definite tissue specificity such as LDH, CPK, Aldolase, ACP and -amylase have been described along with some other diagnostically

TABLE 2: LACTATE DEHYDROGENASE ACTIVITY - PERCENTAGE ACTIVITY DISTRIBUTION IN VARIOUS HUMAN TISSUE (20a).

Organ	Isozyme distribution				
	H_4	H_3M_1	H_2M_2	H_1M_3	M_4
Heart	60	30	5	3	2
Kidney	28	34	21	11	6
Cerebrum	28	32	19	16	5
Liver	0.2	0.8	1	4	94
Sk. muscle	3	4	8	9	76
Skin	0	0	4	17	79
Lung	10	18	28	23	21
Spleen	5	15	31	31	18

TABLE 3: ALDOLASE ACTIVITY IN VARIOUS HUMAN TISSUES (93).

Tissues	Isozyme distribution in u/g of wet tissue (% total activity)		
	A	B	C
1. Sk. muscle	19,600 (97)	-	-
2. Heart	2,068 (90-96)	0-244 (0-10)	0-264 (0-12)
3. Lung	254 (90)	-	-
4. Spleen	470 (96)	-	-
5. Kidney	224 (40)	336 (60)	-
6. Liver	114 (14)	700 (86)	-
7. Small intestine	255 (50)	255 (50)	-
8. Cerebrum	2125 (70)	-	875 (30)
9. Cerebellum	1200 (33)	-	2400 (66)

TABLE 4: CREATINE PHOSPHOKINASE ACTIVITY IN VARIOUS HUMAN TISSUES (94).

Tissues	Isozyme distribution in u/g of wet tissue (% total activity)		
	MM	MB	BB
1. Sk. muscle	3281 (100)	0-623 (0-19)	0 (0)
2. Heart	313 (78)	56-169 (14-42)	0 (0)
3. Brain	0 (0)	0 (0)	157 (100)
4. Colon	4 (3)	1 (1)	143 (96)
5. Stomach	4 (3)	2 (2)	114 (95)
6. Uterus	1 (2)	1 (3)	45 (95)
7. Thyroid	7 (26)	0.3 (1)	21 (73)
8. Lung	5 (35)	0.1 (1)	9 (64)
9. Kidney	2 (8)	0 (0)	19 (92)
10. Prostate	0.3 (3)	0.4 (4)	9.3 (93)
11. Spleen	5 (74)	0 (0)	2 (26)
12. Liver	3.6 (90)	0.2 (6)	0.2 (4)
13. Pancreas	0.4 (14)	0 (1)	2.6 (85)
14. Placenta	1.4 (48)	0.2 (6)	1.4 (46)

TABLE 5: ALKALINE PHOSPHATASE ACTIVITY IN HUMAN TISSUES (95).

Tissues	Activity (u/g of wet tissue)	
	MAP buffer	DEA buffer
1. Adrenal	30.0	66.0
2. Placenta	36.0	-
3. Liver	12.6	27.0
4. Bone	7.5	18.0
5. Spleen	7.5	18.0
6. Lung	6.6	15.0
7. Intestine	4.8	9.0
8. Kidney	4.2	11.0
9. Prostate	3.3	6.6
10. Thyroid	2.1	5.1
11. Heart	1.8	3.6
12. R.B.C.	0.02	-

MAP-2-methyl-2-aminol-propanol buffer DEA-diethylamine buffer.

TABLE 6: TRANSAMINASE ACTIVITY IN HUMAN TISSUES RELATIVE TO SERUM AS UNITY (96).

Tissue	AST	ALT
1. Heart	7800	450
2. Liver	7100	2850
3. Sk. muscle	5000	300
4. Kidney	4500	1200
5. Spleen	700	80
6. Lung	500	45
7. Pancrease	1400	130
8. R.B.C.	15	7
9. Serum	1	1

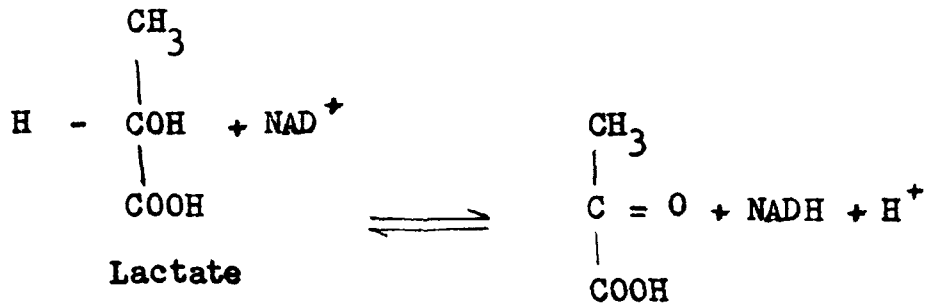
important enzymes with wide tissue distribution in the following pages. Tables 2, 3, 4, 5, and 6 show the activities of some of these enzymes in human tissues.

1. L-lactate dehydrogenase (EC 1.1.1.27):

Cell free extracts able to catalyze the oxidation of lactate to pyruvate were first obtained in 1932(3). The first purified LDH was reported by Straub (4) in 1940.

a) General properties: It has a molecular weight of 140,000 daltons containing four subunits of two different types (H & M) each weighing 35,000 daltons.

It catalyses the reversible oxidation reduction reaction:



Lactate

in the glycolytic cycle.

Pyruvate

The optimum pH and temperature of the enzyme are 7.2 - 9.8 and 32°C. The enzyme does not contain any metal

ions (5.6). No disulphide bridges have been reported for LDH, however, each subunit contains one critical cystein residue, modification of which results in loss of activity (7).

It catalyses i) Ketopyruvate reduction, ii) Glyoxalate oxidation and reduction, iii) cyanide addition to nicotinamide. The equilibrium constant for the reduction of NAD^+ depends on the substrate. The presence of a coenzyme, NADH $\longleftrightarrow$ NAD is essential for the catalytic function of LDH.

Sulfhydryl group appears to be a part of the active site of the enzyme which is strongly inhibited by para-chloro - mercuribenzoate or N-ethylmaleimide. Oxalate inhibits the forward reaction in a noncompetitive manner, while the prodout pyruvate in high concentration becomes competitive inhibitor Oxalate acts as a competitive inhibitor in backward reaction.

It is also inhibited by lactate at a very high concentrations. As with most inhibitors, the H_4 isozyme (K_i = 26 mM) is more sensitive than M_4 isozyme (K_i = 130 mM) (8).

b) <u>Isozymes and tissue specificity</u>: LDH occurs in animal tissues as five differene isozymes separable by electrophoresis. Each isozyme contains four polypeptide chains of two different (H & M) types in different permutation (9, 10).

The difference in isozyme composition in various tissues or in other words the distribution of these two polypeptide chains has been correlated to different metabolic requirements and the oxygen concentration of the tissues (10-13).

Although there are only two major structural genes (corresponding to the M & H chains), there is a complex variety of other LDH genes which can be expressed in some tissues at certain stages of development.

In skeletal muscle the LDH isozyme that predominates contains four M chains and in heart the predominant isozyme contains four H chains. The LDH isozymes in other tissues are a mixture of five possible forms, which may be designated as M_4, M_3H, M_2H_2, MH_3,H_4 or LDH_5, LDH_4, LDH_3, LDH_2 and LDH_1 respectively. LDH X is an isozyme coded by C gene present in mammalian testis.

For all practical purposes, considering the incidence of diseases of different systems, LDH isozyme can be considered as tissue specific and thus helpful in clinical diagnosis.

The major localization of various isozymes is shown in Table 7.

TABLE 7: MAJOR LOCALISATION OF VARIOUS LDH ISOZYMES.

LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
Heart	RBC	Lung	Kidney-	Liver
RBC	RE system	Placenta	Medulla	Sk.muscle
RE system	Kidney-		Sk. muscle	Pancreas
Kidney-	Cortex		Pancreas	Kidney-
Cortex	Lung		Placenta	Medulla

TABLE 8: CLINICAL CONDITIONS ASSOCIATED WITH ELEVATED SERUM LDH LEVEL.

LDH Isozymes	Clinical conditions
LDH_1 and LDH_2	Haemolysis, Myocardial infarction, Renal cortex infarction, some tumours.
LDH_4 and LDH_5	Hepatocellular damage (necrosis, inflammation, congestion), skeletal muscle trauma, Crush injury.
LDH_2, LDH_3 and LDH_4	Malignancies, especially leukemias and lymphomas. Lung Disease and congestion.

The isozymes are distinguishable by their chemical composition and their kinetic and immunological properties.

At pH 8.6, the electrophoretic mobility of LDH, is highest while that of LDH_5 is lowest and remains near origin. Other isozymes remain in between.

The heat stability of LDH isozymes are of diagnostic importance. LDH_5 is heat labile at 65°C for 30 minutes in contrast to heat stability of LDH_1.

LDH_1 has a low K_m for pyruvate and is strongly inhibited by pyruvate whereas LDH_5 has a higher Km for pyruvate, is not inhibited by pyruvate and is more active catalytically.

c) <u>Clinical significance</u>: LDH isozyme fraction is most useful for ruling out myocardial damage and muscle injury occasionally for monitoring progression of certain malignancies.

The normal serum level of LDH by Henry's L-P (14) method is 63-155 U/L in male, 62-131 U/L in female and children having 10-15% higher value. Among this LDH_1 - 18-33%, LDH_2 - 28-40%, LDH_3 - 18-30%, LDH_4 - 6-16%, LDH_5 - 2-13%.

Some of the clinical conditions associated with elevations of different LDH isozymes are summarized in Table 8.

2. Creatine phosphokinase (EC -2.7.3.2):

Creatinine phosphokinase was first crystallized from rabbit skeletal muscle (15). It has also been isolated in pure form from a number of sources.

a) General Properties: It has a molecular weight of 80,000 daltons containing two subunits each weighing 40,000 daltons. It catalyses the reversible phosphate transfer from creatinine phosphate to adenosine diphosphate.

$$\text{Creatinine} + \text{Mg ATP}^{2-} \xrightarrow[\text{pH - 6.8}]{\text{pH -9.0}} \text{Phosphocreatinine}^{2-} + \text{MgADP}^{-} + \text{H}^{+}$$

It s physiological role is associated with ATP generation for contractile or transport systems.

The optimum pH and temperature of the enzyme are 6.8 - 9 and 25°C = 37°C respectively.

The amino acid compositions of MM & BB types are not very different and the BB type contains significantly less of the basic amino acids than the MM forms explaining the greater electrophoretic mobility. Amino acid composition of MB form is intermediate between the two parent forms. It is a thiol enzyme and each subunit contains one catalytic site. The reactivity of essential thiol group is closely

linked to the conformational changes that occur when both substrates are bound to the enzyme.

For the rabbit muscle enzyme Mg^{2+}, Mn^{+2}, Ca^{+2} and CO^{+2} have been found to act as activators while Ba^{2+}, Sr^{2+}, Be^{2+}, Ni^{2+}, Cr^{2+}, Cd^{2+} and Zn^{2+} were inhibitory (16). Anions such as So_4^{-2}, Cl^- and PO_4^{-3} all acted as competitive inhibitors of the enzyme for phosphocreatinine and non competitive inhibitors for Mg ADP (17).

b) Isozymes and tissue specificity: CPK occurs in animal tissues as three different isozymes separable by electrophoresis (18-20). Each isozyme is a dimer composed of two non-identical monomeric subunits, M and B. The enzyme is found primarily in skeletal muscle (maximum concentration), cardiac tissue and brain. Although the majority of isozymes are cytoplasmic, it may also be present in other subcellular fraction particularly mitochondrion. Isozymes of CPK exhibit tissue specificity. Thus the isozyme MM is the predominant form in human skeletal muscle although a small amount is also found in heart in which tissue the predominant form of the enzyme is MB. A small amount of CPK-MB is also present in skeletal muscle. The isozyme BB is found primarily in brain tissue and to a smaller extent in G.I. tract.

Developmental studies show that the BB form is predominant in all early embryonic tissues. However, at adult stage, the expression of the gene for BB is restricted and is associated primarily with the brain and some gut associated tissues.

The various forms of isozymes can be separated by electrophoresis, ion exchange chromatography and immunological techniques.

The anodic mobilities of the various forms of enzymes at pH 8.6 were found to follow the order BB > MB > MM. Infact the movement of isozyme MM at pH 8.6 is hardly perceptible.

c) Clinical Significance: Serum CPK is increased in nearly all patients, when there is injury, inflammation or necrosis of skeletal or heart muscle (20). Decreased level of the enzyme in human serum has little or no clinical significance. The normal serum values of CPK by B MD CK-NAC method (20a) is upto 160 U/L in males and upto 130 U/L in females. Blacks tend to have higher values than white. Serum level can be increased by exercise. Well muscled individuals have normal CPK level, that is higher than those of small sedentary person. The isozyme distribution in the serum of normal individual form one month to adult age is MM - 96-100%, MB - 4% and BB - 0%.

TABLE 9: CLINICAL CONDITIONS ASSOCIATED WITH ELEVATED SERUM CPK LEVELS.

CPK Isozymes	*Clinical conditions*
CPK - MM	Extreme physical exertion, physical and surgical trauma, Intramuscular injections, Rhabdomyolysis, Malignant hyperpyrexia (MM, BB). Myopathies except dernervation myopathy (MM, MB), certain infectious diseases that affect muscle.
CPK - MB	Myocardial infarction, muscular dystrophy especially Duchenne's type, Polymyositis.
CPK - BB	Malignancy, prostatic, pulmonary and neurological disorders.
CPK -	Hypothyroidism, Schizophrenia, myocarditis, pericarditis, coma.

Some of the clinical conditions associated with the elevations of different CPK isozymes are summarised in Table 9 .

3. Aldolase (EC - 4.1.2.13).

In 1934 Meyerhof and Lohmann discovered the reversible cleavage of hexosediphosphate to form 2 moles of dihydroxy-acetone phosphate, catalysed by an enzyme which they called zymohexase (21). They later renamed it aldolase when they found that it would catalyse the reversible condensation of dihydroxyacetone phosphate with a variety of aldehyde (22,23). Aldolase was crystallized from rat muscle by Warburg and Christian (24).

a) General Properties: It has a molecular weight of approximately 160,000 daltons, containing four subunits, each having a molecular weight of 40,000 daltons.

It catalyses the reversible reaction:

$$\text{Fructose-16 diphosphate (FDP)} \xleftarrow{\text{EC 4.1.2.13.}} \text{dihydroxyacetone} + \text{Glyceraldehyde-3-phosphate.}$$

The optimum pH and temperature of the enzyme are 6.8-7.2 (25) and 37°C, respectively. Class I aldolases do not require metal ion as cofactor while class II aldolases

require it. Molecular weight and subunit structure of aldolase A & B are almost similar (26, 27). The amino acid composition and sequence at the active site of the three is closely homologus though they differ significantly in the other regions of the molecule.

Aldolase B differs in catalytic and immunologic properties from aldolase A (27-29) whereas catalytic property of aldolase C is found to be similar to that of aldolase. Antibodies prepared against each type of subunit is specific and does not cross react with the other type (28). Monomers in the hybrid forms act independently without subunit interaction and antibody against one subunit in a heteropolymer can cause total inhibition of the tetrameric enzyme.

Aldolase B is inhibited by AMP > ADP but not by by ATP whereas aldolase A is competitively inhibited by all three nucleotides, the order being ATP > ADP > AMP. Aldehydes also inhibit the enzyme.

b) Isozyme and tissue specificity: Two classes of aldolases are present. Class I aldolases which form schiff base intermediate with the substrate, are widely distributed in mammals and higher plants. Class II aldolases are metalloaldolases and do not form schiff base and resemble yeast aldolase present in yeast, bacteria and fungi.

Rutter and his co-workers (30-32) have identified three distinct aldolase isozymes based on their electrophoretic properties FDP/FIP activity ratios and inhibition yby specific antibodies in tissues of mammals and other vertebrates.

It is a tetramer composed of two of three known subunits designated as A, B and C. Aldolase A is the major form, usually the only form present in skeletal muscle. Aldolase B, the predominant form in liver and kidney, aldolase C is present in brain together with aldolase A. In tissues where more than one aldolase isozyme is present, hybrid forms composed of subunits of both primary forms are also found to be present. The hybrid isozyme composed of three A subunits and one C subunit is present in low concentration in serum as the primary isozyme in normal serum is a homomer. Aldolase A is primarily present in skeletal muscle and to some extent in heart and brain. Aldolase B is mainly present in liver and a small amount in kidney and small intestine. Brain is the predominant source of aldolase, though a small amount is also present in heart. When analysed as homotetramers, aldolase A, B, C

Aldolase A	Aldolase B	Aldolase C
Skeletal muscle	Liver	Brain
Heart	Kidney	Heart
Brain	Small Intestine	

have similar pH activity profiles, K_m for FDP and molecular weights. The ratio of the FDP/F-IP cleavage rate is respectively, 50 for aldolase A, 1-2 for aldolase B and 26 for aldolase C. Aldolase A is usually present in all the human embryonic tissues, which are replaced subsequently after birth. The transition form A to B occurs early in liver (Before day 55) and later in kidney (33). Aldolase C begins to appear in human brain after day 100. Human embryonic heart muscle contains aldolase A and C together with hybrid forms; Traces of these hybrid forms persist in adult life.

c) Clinical significance: Though not frequently measured, it has a definite significance in some skeletal muscle disorders (33a). The normal serum level of aldolase by the method of Bergmeyer and Bernt (20a) is 2.61-5.71 U/L in males and 1.98-5.54 U/L in females. The children has twice the adult level.

Aldolase level is mainly increased in skeletal muscle disorder except denervation myopathy and mesthenia group (aldolase A), liver necrosis (aldolase B), myocardial necrosis (aldolase A), hepatoma (aldolase A) and haemolysis (34, 35).

4. Acid Phosphatase (E.C. - 3.1.3.2):

In 1924 Martland et al. (36) reported phosphatase activity in RBC. While studying the source of acid phosphatase activity in male urine, Kutscher and Wolberg discovered the very high activity of ACP in human prostate (37).

a) General Properties: It has a molecular weight of about 100,000 daltons but varies considerably with isozymes. It is non specific phosphatases that catalyse the hydrolysis of orthophosphoric mono ester to an alcohol and phosphate group at an acid pH.

$$\underset{\text{organic ester}}{R_1\text{-}O\text{-}PO_3H_2} + \underset{\text{phosphate alcohol}}{H\text{-}OH} \xrightarrow[pH\ 5]{ACP} \underset{\text{alcohol (new)}}{R_1\text{-}OH} + \underset{\text{phosphate (ester new)}}{H\text{-}O\text{-}PO_3H_2}$$

The optimum pH ranges from 4.8-6.0 depending on tissue source and substrate used. Optimum temperature is 37°C.

Phosphoryl transfer may be effected by prostatic ACP to acceptors other than solvent (38-40). It is very sensitive to surface inactivation. A variety of agents gelatin, BSA, egg albumin and tween-80 protect the enzyme against inactivation. It is inhibited by alpha hydroxy carboxylic acid and also by fluoride at a relatively low concentration. Prostatic ACP is partially and reversibly inactivated by Ca^{+2} ion.

Anions such as chloride, bromide and thiocyanate inhibit prostatic ACP activity both competitive and non-competitively.

Behrendt (41) shows that RBC enzyme is much more active than serum ACP in splitting phenyl phosphate. RBC ACP splits neither alpha nor beta naphthyl phosphate. Different metal ions show differences in inhibition between RBC and prostatic ACP.

b) Isozymes and tissue specificity: Using disc acrylamide electrophoresis of serum at pH 5, different bands have been found and named as ACP , ACP_1, ACP_2, ACP_3, ACP_{3b}, ACP_4 and ACP_5 according to their relative mobility in the electric field.

ACP is present in very high concentration in prostate RBC, WBC, platelet and to a lesser extent in liver, spleen, bone, kidney (42-47). It is a lysosomal enzyme. Clinically in most cases, highly raised level of ACP indicates advanced stage of prostatic malignancy, haemolysis or platelet destruction. Thus it is mainly specffic or prostate, RBC and platelet. Tissue specific isozymes can be differentiated by assaying the enzyme in following manner (48-50).

The activity of isozymes from prostate liver, kidney and spleen is substantially inhibited (70-95%) by L-tartarate.

The prostatic, splenic, hepatic and renal isozymes are not inhibited by formaldehyde which inhibits isozyme from RBC. Ethanol inhibits prostatic and RBC ACP but not ACP from liver and spleen. The prostatic ACP activity can be specifically monitored using a new substrate namely thymolphthalein monophosphate. This method is used in the diagnosis of prostatic diseases (51).

The isozyme pattern of ACP in various organs show significant differences. As many as twenty different isozymes have been reported (52- 53) due to heterogeneity in carbohydrate portion of molecule.

Of these isozymes, only prostatic ACP (ACP_2, PAP) and isozymes 1 and 5 from human spleen have been used for clinical diagnosis. The elevated level of isozymes 1 and 5 in human spleen is suggestive of Gaucher's disease.

In the serum, the contribution of ACP activity comes from platelet and RBC mainly and to a lower extent from prostate.

c) Clinical significance: The normal level of the enzyme is 1-3.5 KA/dl by the method of King and Jegatheesan (101). Prostatic ACP level is usually less than 0.8 KA/dl. A physiological elevation of this enzyme occurs in children

and during pregnancy. Determination of serum PAP is an useful procedure primarily in the assessment of carcinoma of prostate with or without metastasis (54-57). PAP fraction is also increased in prostatic infarction and operative trauma to prostate (58). In idiopathic thrombocytopenic purpura serum ACP may be increased (59, 60).

Gaucher's disease, Niemann pick disease, various bone diseases such as Paget's disease (61), metastatic carcinoma of bone, multiple myeloma may be associated with raised ACP. Rarely ACP may be elevated in liver and kidney diseases. The detection of high activities of ACP in vaginal washings indicates the presence of seminal fluid. Decreased serum ACP has no clinical significance.

5. Alpha Amylase (E.C. - 3.2.1.1):

Alpha amylases were named by Kuhn (62) because the hydrolytic products possess the alpha configuration. The prefix alpha or beta conveys information about product streochemistry not substrate specificity.

a) General Properties: It has a molecular weight of 40,000-50,000 daltons. It degrades complex carbohydrate molecules into smallercomponents. Human amylases are alpha amylases. They

are endo-amylases and hydrolyze the internal 1,4-glycosidic bonds of polysaccharides in a random manner.

The optimum pH and temperature of the enzyme are 7 and 37-40°C respectively. The diffusion coefficient and partial specific volume of pancreatic and salivary enzymes are almost similar. There are minor changes in the amino acid composition of these proteins.

It is a metallo enzyme as it contains Ca^{++} (63-65). It s removal results in both reversible (63, 64) and irreversible inactivation or in great loss of thermal stability. Ca^{++} deficient amylase is found to be susceptible to protease digestion (67). Ca^{++} stabilizes the compact architecture of the molecule and it helps to maintain an enzymatically active conformation (64). Ca^{+2} and Mg^{+2} are activators at low concentration but become inhibitors at high concentration ($>$ 10 mM). EDTA inhibits about 90% of enzyme activity. Halide ions are required for enzyme activity. The order of their effectiveness is Cl $>$ Br $>$ I $>$ F. Heavy metals (63) like mercury, silver, copper and lead inhibit amylase. Ammonium molybdate and ascorbic acid (63,66) also inhibit amylases. It is speculated to be due to sulfhydryl modification (67a).

b) <u>Isozymes and tissue specificity</u>: The enzyme is present at it's highest concentration in pancreas and salivary gland and

can be considered specific of these tissues. Other tissues such as liver, fallopian tubes, certain tumours and skeletal muscle also contain amylase (68-70) to a minor extent.

It has been found that liver is the major source of serum amylase (71); though the tissue origin of amylase in normal serum is still detectable.

Amylases from a given organ in different species often display greater similarity in their enzyme behaviour than amylases from different organs of the same species. The salivary pancreatic and serum amylases also differ electrophoretically (72,73). At least seven bands can be found on electrophoresis from human tissue sources of the enzyme. Since the amylases from diverse sources have the same approximate molecular weight and are dimeric hydridiztion of two genetically controlled monomers is hypothesized to explain the three bands, usually observed in many higher animal species (74). On electrophoresis of serum amylase, three slower moving bands are identified as pancreatic amylase (P_1-P_3) and three faster moving bands are identified as salivary amylase (S_1-S_3). S_1 and P_3 isozymes are most commonly present in the population. Pancreatic amylase is more heat sensitive than the normal serum amylase.

c) Clinical significance: The normal level of alpha amylase is less than 1500 u/l in serum and less than 500 u/24 hours in urine by iodometric method of caraway and Wendell (102).

It is principally measured for the diagnosis of acute pancreatitis. It is also elevated in some other conditions like parotid gland disease, mumps, parotitis, calculi in parotid duct, perforated peptic ulcer, intestinal obstruction, renal failure, ectopic pregnancy, macroamylasemia, haemolysis, spasm of spincter of oddi by drugs like morphine and codeine, chronic liver disease.

6. Transaminases:

a) General Properties:

i) Aspartate aminotransferase (E.C. 2.6.1.1.): It is an enzyme that catalyzes the transfer of an amino group from specific amino acids (L-glutamate or L-aspartate) to specific keto-acids (Alpha ketoglutarate or oxalo acetate). It has a molecular weight of 110,000 daltons containing two subunits of equal size. The optimum pH and temperature of the enzyme are 7.4 and 37°C respectively. The reaction catalyzed by AST is freely reversible and has an equilibrium constant of about 1.0.

$$\text{L-Aspartate} + \alpha\text{Ketoglutarate} \underset{\longleftarrow}{\overset{\text{AST}}{\longrightarrow}} \text{Oxaloacetate} + \text{Glutamine}$$

AST has pyridoxal phosphate as it s prosthetic group (coenzyme) which is responsible for carrying the amino group via Schiff's base formation. Although at phyriological pH the reaction is energetically favoured toward the formation of L-aspartate and α-ketoglutarate (to the left), in vivo the reaction goes to the right to provide a source for nitrogen of the urea cycle. The glutamate thus produced is deaminated by glutamate dehydrogenase resulting in ammonia and regeneration of α-ketoglutarate.

The greatest amount of AST is present in the cardiac tissue followed by lesser amounts in liver, skeletal muscle and kidney. A small amount is present in the pancreas, spleen, lung, erythrocytes, skin and serum. Despite wide tissue distribution, AST is usually specific of heart and liver. It is present in both the mitochondria and cytoplasm of the cell. Various observers have discrened that species differences are present (33a).

(ii) Alanine aminotransferase (E.C. 2.6.1.2.): It is an enzyme that catalyses the transfer of an amino group from specific amino acids (L-glutamate or L-Alanine) to specific keto acids (α-ketoglutarate or pyruvate).

It has a molecular weight of 180,000 daltons. The optimum pH and temperature are the same as found for AST. The substrate specificity, coenzyme and mechanism of reaction are same as in AST except that one of the amino acids is L-Alanine instead of L-aspartate and one of the ketoacids is pyruvate instead of oxaloacetate.

$$\text{L-Alanine} + \alpha\text{-ketoglutarate} \overset{\text{ALT}}{\rightleftharpoons} \text{Pyruvate} + \text{L-glutamate.}$$

Here also the chemical equilibrium favours the reaction to the left. In vivo this reaction goes to the right to provide a source of nitrogen for urea cycle. The pyruvate thus generated is available for entry into TCA cycle, whereas the glutamate is deaminated yielding ammonia and alpha ketoglutarate.

ALT is found in greatest amounts in liver with lesser amounts in kidney, heart and various other tissues. Practically it can be regarded specific for liver. It is a cytosolic enzyme. Species differences have also been noted(33).

b) <u>Isozymes</u>: Electrophoretic separation of serum as well as liver and heart muscle AST on paper usually shows the enzyme activity in two fractions (75,76). Separation on starch gel appears to yield two to five components (77,78).

The main distinction appears to be between the cytoplasmic enzyme and the mitochondrial enzyme (79-83). They differ in physical and chemical properties and amino acid sequence. The isozymes are not organ specific (84). The clinical usefulness of the study of this isozyme is not so well established.

c) Clinical significance: The normal serum values range from 5-20 iu/l and 5-15 iu/l for AST and ALT respectively by the method of Reitman and Frankel (99). The commonest causes for raised serum transaminase levels are heart and liver diseases. AST level rises in acute myocardial infarction but not in angina pectoris (85,86). Persistent and recurrent elevation of AST indicates extension of infarct. A value above 1500 IU/L is a bad prognostic sign. A ratio of AST/ALT is helpful in determining the source and aetiology of transaminase elevation. In myocardial disease it is greater than 1, while it is less than 1 in hepatic disease. Both AST and ALT are elevated in liver damage such as occurs in neoplastic, inflammatory or degenerative lesions.

In acute hepatitis, ALT level is greater than AST. In severe toxic necrosis of liver or severe viral hepatitis, AST level is much greater than ALT. In cirrhosis of liver, moderate rise of AST occurs.

AST level is also elevated in infectious monomucleosis dermatomyositis, renal and pulmonary infarction, burn and shock.

7. Alkaline phosphatase (E.C. 3.1.3.1.)

The nonspecific phosphatases that effect the hydrolysis of phosphomonoesters to alcohol and inorganic phosphate at an alkaline pH are designated as alkaline phosphatase.

a) General Properties: It has a molecular weight of about 120,000 daltons but varies considerably with tissue source of enzyme. The optimum pH ranges from 8.6-10.3 depending on the type of substrate (87), the substrate concentration, incubation temperature and the tissue source of the enzyme (88). The optimum temperature is 37°C.

The enzyme catalyses the reaction:-

$$\underset{\text{Phosphomonoester}}{R_1 - PO_4} + H_2O \xrightarrow[pH\ 8.6\ Mg_2]{\text{Alk. phosph.}} \underset{\text{Alcohol}}{R - OH} + \underset{\text{Inorganic phosphate}}{PO_4}$$

It can act as phosphotransferases too. It s activity is dependent on Mg^{2+} for activation but is inhibited by Ca^{+2} (89).

and phosphate ions (89). Glucose and glycerol increase the activity by acting as phosphate acceptors (89). Diminution of activity with oxalate (89), citrate (89) and EDTA (89) may be due to Mg^{+2} chelation by these agents.

Ammonium hydroxide, borate or glycine based buffers have an inhibitory effect on the enzyme activity, whereas in 2-amino-2-methyl-1-propranol buffer significantly higher activity was found (89);

b) Isozymes and tissue specificity:- Isozymes of ALP from bone, liver, intestine, placentra and kidney have been identified in the blood of individual patients. Regan and Nagao type of ALP were discovered in the sera of certain percentage of cancer patients. The Regan and Nagao type of isozymes are very similar to placental type ALP in physicochemical and immunological properties.

Electrophoresis, differential heat sensitivity and differential chemical inhibition are currently the methods of analysis most commonly used. Both liver and intestinal ALP are moderately heat stable at 56°C for 15 minutes. Intestinal ALP is inhibited by phenyl alanine unlike liver ALP. Placental, Regan and Nagao type of ALP are heat stable at 56°C for 15 minutes and also inhibited by phenyl alanine. Bone ALP is heat labile and not inhibited by phenyl alanine. Normal

liver (Liver I) isozyme migrates fastest while bone and intestinal isozymes migrate progressively slower. The electrophoretic detection of tissue source of serum isozymes in various clinical states has not been successful (90). Intestinal and liver II isozyme (intracellular) are not normally present in serum. Regan isozyme is produced by neoplastic cells such as bronchogenic carcinoma, ovarian tumour etc.

Inspite of wide tissue distribution, the enzyme is mainly specific of liver and bone. In growing individual, it is present in greatest concentration in the bone. In the adult, liver and intestine contain the largest amount of enzyme. Kidney and lung also have a large amount of enzyme. Other sites are placenta, vascular endothelium, bile duct, epithelium, thyroid epithelium and peripheral blood. The enzyme is present on the cell membrane.

c) Clinical significance: The normal level is 3-13 KA/dl by the method of Kind and King (100). A physiological increase in the level occurs in growing children and during 2nd and 3rd trimester of pregnancy. Men usually have a higher value than women. The common causes of it's elevation are extra-hepatic biliary obstruction and osteomalacia of bone or other

osteoblastic bony lesions. The bone ALP will increase in growing children, secondary hyperparathyroidism, osteoblastic metastasis, Paget's disease and healing bone fracture.

Liver ALP is noted in extra and intrahepatic cholestasis. Common causes of raised liver ALP are cirrhosis, metastatic liver, space occupying lesion of liver. Placental ALP is noted during pregnancy. The tumour (Regan) enzyme can be seen in malignancy (33).

Intestinal ALP is seldom found in large amount in peptic ulcer and ulcerative colitis (91, 92). Similarly splenic diseases, renal and pulmonary infarction may give rise to elevated level of respective tissue isozymes.

Pathological conditions causing tissue damage result in the release of cellular enzymes in the circulatory system (96). The elevated level of the serum enzymes that are specific for a particular tissue will obviously have a great diagnostic value for the study of the pathology of that tissue. Unfortunately it is not always possible to estimate the enzymes in human tissues. However, as various metabolic patterns of mammalian systems do not differ substantially at least qualitatively, extrapolation of data on enzyme levels obtained from tissues of other mammals can be extrapolated to human. As the cellular levels of enzymes critically depend,

among others, on the levels of tissue proteinases and their inhibitors, it will be advisable to determine these parameters along with the concentration of enzymes. For comparison, normal values of serum enzymes must be determined on statistically adequate sample size of a given population. Since the normal values of serum enzymes usually show strong dependence on nutritional status, age, sex and ethnic groups, their determination in a particular population will be useful especially in a country like India where these values change significantly from one population group to another. In view of these considerations, we have determined the levels of transaminases, alkaline phosphatase, acid phosphatase and amylase in tissues of goat buffalo, rabbit and human. The normal levels of these enzymes in blood samples of Aligarh population were investigated. The sample size chosen in this study comprises of two hundred invididuals of different age and sex. The results along with their analysis and discussion are presented in this thesis.


EXPERIMENTAL

II. EXPERIMENTAL

A. Materials

1. Reagents used for ammonium sulphate fractionation of tissue homogenate: Ammonium sulphate was obtained from Sarabhai chemicals, Baroda. Sephadex G-25 was purchased from Pharmacia Fine Chemicals Uppsala, Sweden.

2. Reagents used for enzyme assay: Reagents used for the estimation of transaminase activity were DL aspartic acid (B.D.H., England), α-ketoglutaric acid (Fluka, Switzerland), alanine (Sisco, Laboratories, Bombay), sodium pyruvate (Loba Chemie Indoaustranol Co., Bombay), 2,4-dinitrophenyl hydrazine (Loba Chemie Indoaustranol Co., Bombay).

Disodium phosphate (B.D.H., England), 4-amino antipyrin (B.D.H., England), potassium ferricyanide (Loba Chemic Indoaustranol Co,, Bombay) and Phenol (B.D.H., India) were used in the estimation of alkaline phosphatase activity. Reagents used for estimation of acid phosphatase activity were citric acid (Sarabhai Chemicals, Baroda), L(+) tartaric acid (Sarabhai Chemicals, Baroda). Others were as in alkaline phosphatase.

Benzoic acid (Sarabhai Chemicals, Baroda), soluble starch (E. Merck, India), potassium iodide (B.D.H., India),

sublime iodine (E. Merck, Germany) and potassium fluoride (B.D.H., India) were used in the determination of amylase activity.

3. Reagents used for estimation of protein: Reagents used for estimation of protein were Na-K tartarate (Glaxo Laboratories), copper sulphate (Sarabhai Chemicals, Baroda), sodium tungstate (B.D.H., India), sodium molybdate (Veb Gen Pharm, Germany), Lithium sulphate (B.D.H., India), orthophosphoric acid (B.D.H., India), liquid bromine.

4. Others reagents used: Other reagents used were disodium hydrogen phosphate (Sarabhai Chemicals, Baroda). Potassium dihydrogen phosphate (Merck, India), sodium hydroxide (B.D.H. , India), sodium carbonate (Glaxo Laboratories), sodium bicarbonate (B.D.H., India), hydrochloric acid (Sarabhai Chemicals, Baroda).

All glass double distilled water was used throughout.

B. Methods

1. pH measurement: pH of solution was measured either on an Elico LI-10 pH meter using Elico glass and calomel electrodes or with EC combination electrode. The pH meter was calibrated

with 0.05 M potassium hydrogen phthalate buffer, pH 4.0, at 25°C in an acidic pH range and with 0.01M sodium tetraborate buffer, pH 9.2, at 25°C in the basic pH range.

2. Optical measurement: Absorbance of solutions in the visible range was determined on photochem colorimeter, model C-110.

3. Determination of protein concentration: Protein concentration was measured by the method of Lowry et al. (97) using bovine serum albumin as standard.

Folin-phenol reagent was prepared according to the method of Folin and Ciocalteu (98). The copper reagent was prepared by mixing 4% sodoium carbobate, 2% sodium-potassium tartarate and 2% copper sulphate in the ratio of 100: 1: 1 in the sequence to avoid precipitation.

To 1 ml of protein solution, 5 ml of copper reagent was added. After 10 minutes 1 ml of Folin-phenol reagent was added and the solution was kept for 30 minutes for the development of the colour. Colour intensity was read at 650 or 700 nm against a blank in which instead of protein solution, 1 ml water or buffer was taken.

The protein concentration of the solution was then

determined from the calibration curve obtained with bovine serum albumin.

4. Assay of transaminases: The activity of transaminase was measured by the method of Reitman and Frankel (99).

a) Principle:- The pyruvate produced by transamination by ALT reacts with 2,4 dinitrophenyl hydrazine (DNPH) to give a brown coloured hydrazone, which is measured in colorimeter at 510 nm. The oxaloacetate formed in the reaction with AST decarboxylates spontaneously to pyruvate which is again measured by hydrazone formation.

b) Reagents: i) 0.01 M phosphate buffer pH 7.4; ii) AST substrate (200 mM-DL-aspartic acid; 2 mM alpha ketoglutarate) - For the preparation of AST substrate 13.3 g of DL aspartic acid was dissolved in minimum amount of 1 N sodium hydroxide and the pH was adjusted to 7.4 by addition of sodium hydroxide. Now 0.146 of alpha Ketoglutaric acid was dissolved in it and the pH was again adjusted to 7.4 with 1 N sodium hydroxide. Finally the volume was made upto 500 ml with phosphate buffer. It was stored frozen at -15°C.

iii) ALT substrate (200 mM alanine; 2 mM alpha ketoglutarate) - For the preparation of ALT substrate 9 g alanine was dissolved in 90 ml water with the addition of about 2.5 ml of 1 N sodium

hydroxide to adjust the pH to 7.4. Now 0.146 g of alpha keto-glutaric acid was dissolved in it and the pH was adjusted to 7.4 with 1 N sodium hydroxide. Finally the volume was made upto 500 ml with phosphate buffer. It was stored frozen at -15°C.

iv) Stock pyruvate standard:(20 mM) - For the preparation of stock pyruvate standard 220 mg of sodium pyruvate was dissolved in 100 ml of phosphate buffer. It was stored at -15°C in 1 ml aliquots.

v) Working pyruvate standard (4 mM).

vi) 2-4, dinitrophenyl hydrazine (1 mM) - For the preparation of 2-4-DNPH, 19.8 g of 2,4 DNPH was dissolved in 10 ml concentrated hydrochloric acid and was made upto 100 ml with water. It was stored in a brown bottle at room temperature.

vii) 0.4 N sodium hydroxide.

c) Method for estimation of AST: To 0.5 ml of the substrate (previously warmed at 37°C for 3 minutes) was added 0.1 ml of sample, mixed gently and incubated for 60 minutes at 37°C and then 0.5 ml of DNPH solution was added with gentle mixing. After incubation for 20 minutes at room temperature, 5 ml of 0.4 N sodium hydroxide was added. This was incubated for another 10 minutes and the colour intensity was measured at

510 nm. To prepare a control, sample was added after the addition of DNPH. For the preparation of blank, water was added instead of sample. For the preparation of standard, 0.1 ml of working pyruvate standard was mixed with 0.4 ml of substrate and 0.1 ml of water and the rest is similar to test sample.

(d) Method for estimation of ALT: It was same as for AST determination except for using ALT substrate and reducing the incubation time to 30 minutes.

(e) Calculation: The specific activity of the enzyme was calculated from the following formula:

$$\text{Specific activity} = \frac{\text{Optical density test} - \text{Optical density control}}{\text{Time of incubation (hr).} \times \text{Protein concentration of sample (mg)}}$$

The enzyme activity in serum can also be calculated in the following way.

The pyruvate formed by the sample was responsible for the difference between the optical density of test and control ($OD_T - OD_C$).

The pyruvate in 0.1 ml of the working standard (0.4 umole) produced the difference between the optical density of

standard and blank ($OD_S - OD_B$). So the pyruvate formed in 60 minutes by AST with 0.1 ml of samples was:

$$\frac{OD_T - OD_C}{OD_S - OD_B} \times 0.4 \text{ mole.}$$

Thus the pyruvate formed per minute per litre of sample by AST was :

$$\frac{OD_T - OD_C}{OD_S - OD_B} \times 0.4 \times \frac{1}{60} \times \frac{1000}{0.1}$$

$$= \frac{OD_T - OD_C}{OD_S - OD_B} \times 67 \text{ umole.}$$

Similarly for ALT, the pyruvate formed per minute per litre of sample was:

$$\frac{OD_T - OD_C}{OD_S - OD_B} \times 0.4 \times \frac{1}{30} \times \frac{1000}{0.1} = \frac{OD_T - OD_C}{OD_X - OD_B} \times 133 \text{ umole}$$

In both the cases, the calculated pyruvate was converted into I.U./L from the chart given by Wooton (107).

5. Assay of alkaline phosphatase: The activity of alkaline phosphatase was measured by the method of Kind and King (100).

(a) Principle: Phenol released by enzymatic hydrolysis from phenyl phosphate, under defined conditions of time, temperature and pH is estimated colorimetrically.

(b) Reagents:

i) Carbonate buffer pH - 9.9.

ii) Substrate (0.01 mole/L disodium phenyl phosphate): for the preparation of the substrate 2.18 g of disodium phenyl phosphate was dissolved in 1 L of water. The solution was boiled quicklytoto kill any organisms followed by rapid cooling. It was preserved with a little chloroform at $4^{o}C$.

iii) Stock phenol standard (1 mg/ml) - For the preparation of stock phenol standard, 1 g pure crystalline phenol was dissolved in 1 litre 0.1 mol/L hydrochloric acid. It was kept at $4^{o}C$ in a brown bottle.

iv) Working phenol standard (1 mg/100 ml).

v) Sodium hydroxide (0.5 mole/L)

vi) Sodium bicarbonate (0.5 mole/L)

vii) 4-amino antipyrine - For the preparation of 4-amino-antipyrine solution, 6 g of it was dissolved in a litre of water. It was stored in a brown bottle at room temperature.

viii) Potassium ferricyanide: For the preparation of potassium ferricyanide solution, 24 g of it was dissolved in a litre of water. It was also stored in a brown bottle at room temperature.

c) Method: To a mixture of 1 ml buffer and 1 ml substrate (previously warmed at 37°C for 3 minutes), 0.1 ml sample was added, mixed gently and incubated exactly for 15 minutes at 37°C and then 0.8 ml of sodium hydroxide (0.5 mol/L) was added with gentle mixing.

To it, 1.2 ml of sodium bicarbonate (0.5 mol/L) was then added followed by addition of 1 ml of 4-amino-antipyrine solution and 1 ml of potassium ferricyanide solution in sequence with proper mixing after each addition.

The reddish brown colour was compared immediately at 510 nm. To prepare the control, sample was added after the addition of sodium hydroxide. Blank was prepared by taking 1.1 ml buffer with 1 ml of water and the rest is similar to test sample. For the preparation of standard 1.1 ml of buffer was mixed with 1 ml phenol standard (1 mg/dl) and the rest is similar to test sample.

(d) Calculation: The specific activity of the enzyme was calculated by the formula as in the transaminases.

The enzyme activity in serum can also be calculated in the following way.

The amount of phenol present in the standard tube was 10 µg. Thus the phenol produced in 15 minutes in the test was

$\frac{OD_T - OD_C}{OD_S - OD_B}$ X 10 µg, Hence 100 ml of sample would liberate $\frac{OD_T - OD_C}{OD_S - OD_B}$ X 10 mg of phenol. Since 1 KA is the production of 1 mg of phenol in 15 minutes under the conditions of the test.

Alkaline phosphatase activity (KA/dl) $= \frac{OD_T - OD_C}{OD_S - OD_B} \times 10$

6. Assay of acid phosphatase: The activity of acid phosphatase was determined by the method of King and Jegatheesan (101).

(a) Principle:- It is same as described in alkaline phosphatase.

(b) Reagents: All are same as in alkaline phosphatase except the buffer and the tartarate.

i) Citrate buffer (pH 4.9): For the preparation of citrate buffer, 42 g of crystalline citric acid was dissolved in water. To it 376 ml of 1 mol/L sodium hydroxide was added and made upto 1 litre with water. pH was checked and prepared with a few drops of chloroform at 4°C.

ii) Tartarate solution (1 mol/L): For the preparation of tartarate solution, 15 g of tartaric acid was dissolved in

about 70 ml of water. 18.5 ml of 10 mol/L sodium hydroxide was then added and the pH was adjusted to 4.9. Finally the volume was made upto 100 ml with water. It was stored in a dropping bottle at 4°C.

c) Method:- For total acid phosphatase, 1 ml of citrate buffer was mixed with 1 ml of substrate in a test tube and placed in an incubator at 37°C for 3 minutes. Then 0.2 ml of sample was added, mixed gently and incubated for 1 hour exactly at 37°C and then 1 ml of 0.5 mol/l sodium hydroxide was added.

To it, 1 ml of 0.5 mole/L sodium bicarbonate was added followed by addition of 1 ml of 4-amino antipyrine and 1 ml of potassium ferricyanide solution in sequence with proper mixing after each addition.

The reddish brown colour was then compared immediately at 510 nm.

For the determination of the prostatic fraction (tartarate labile), a second tube was prepared exactly in the same manner except for adding a drop of tartarate solution (1 mole/L) before pipetting the serum. To prepare the control, sample was added after the addition of sodium hydroxide.

Blank was prepared by taking 1.2 ml buffer with 1 ml of water and the rest is similar to test sample. For the

preparation of standard, 1.2 ml of buffer was mixed with 1 ml of phenol standard (1 mg/dl) and the rest is similar to test sample.

d) Calculation: The specific activity of the enzyme was calculated by the formula as in the transaminase. The enzyme activity in the serum can also be calculated in the following way.

The amount of phenol present in the standard tube was 10 µg. Thus the phenol is the test was

$$\frac{OD_T - OD_C}{OD_S - OD_B} \times 5 \text{ mg of phenol.}$$

Since 1 KA unit is the production of 1 mg of phenol in 60 minutes under the conditions of the test.

$$\text{Acid phosphatase activity (KA/dl)} = \frac{OD_T - OD_C}{OD_S - OD_B} \times 5$$

To obtain the tartarate labile phosphate, the above formula was used to calculate the results of the two tests (one without and the other with added tartarate solution). The difference between the results represented the phosphatase which had been inactivated by tartarate.

7. Assay of amylase:- The activity of amylase was measured by the iodometric method of Caraway and Wendell (102).

(a) Principle: Starch forms a blue colloidal complex with iodine in solution and the intensity of the colour is directly proportional to the concentration of the starch. The blue colour produced by the starch substrate when combined with iodine, is measured after incubation with sample and compared to a control. The decrease in colour is proportional to the amylase activity.

(b) Reagents:- i) Buffered starch substrate (pH 7):- For the preparation of buffered starch substrate 13.3 g of dry anhydrous disodium hydrogen phosphate and 4.3 g benzoic acid were dissolved in 250 ml water. It was brought to boiling. A separate solution was made with 0.200 g of soluble starch in 5 ml of cold water in a beaker and was added to the boiling mixture, rinsing the beaker out with additional cold water. Boiling was continued for 1 minutes followed by cooling to room temperature and diluted to 500 ml with water finally. It was stored at $4^{o}C$.

ii) Stock Iodine Solution (0.1 N):- To prepare stock iodine solution, 13.5 g of pure sublimed iodine was dissolved in a solution of 24 g of potassium iodide in about 100 ml water

and the final volume was made upto 1 litre with water.

iii) Working iodine solution (0.01 N):- For it's preparation 50 g of potassium fluoride was dissolved in a little water. To it, 100 ml of stock iodine solution was added and the final volume was made upto 1 litre with water. It was stored in a brown bottle at 4°C.

c) Method:- The sample was diluted 1 in 10 with 0.9% saline. To 1 ml of buffered starch substrate (previously warmed at 37°C for 3 minutes), 0.1 ml of the diluted sample was added, mixed gently and incubated for exactly 15 minutes at 37°C. Now 0.4 ml of working iodine solution was added, mixed well followed by addition of 8.5 ml of water with proper mixing. For the control 1 ml of buffered substrate, 8.6 ml of water and 0.4 ml of iodine were mixed in sequence.

The colours were compared immediately at 660 nm using water as a blank.

d) Calculations:- The specific activity of the enzyme was calculated by the formula as in the transaminases.

The activity of the enzyme in serum can also be calculated in the following way. The control tube contained 0.4 mg starch. The amount of starch which had been digested was therefore:

$$\frac{OD_C - OD_T}{OD_C - OD_B} \times 0.4 \text{ mg}$$

The amylase unit is the amount of enzyme digesting 5 mg of starch in these conditions. The amount of enzyme present in 0.01 ml of the sample was:

$$\frac{OD_C - OD_T}{OD_C - OD_B} \times 0.4/5 \text{ units, thus}$$

$$\text{Amylase activity (units/dl)} = \frac{OD_C - OD_T}{OD_C - OD_B} \times 0.4/5 \times 100/0.01$$

$$= \frac{OD_C - OD_T}{OD_C - OD_B} \times 800$$

8. Extraction of protein from various tissues for enzyme assay:

Protein was extracted from liver, kidney, skeletal muscle, heart muscle, brain and lung tissues of buffalo, goat and rabbit.

Each tissue (20 g) was first washed with ice cold water and then peripheral membrane and connective tissues were removed followed by cutting into small pieces. It was then homogenized in phosphate buffered saline in a homogenizer for 10-15 minutes. The homogenate was centrifuged at 7,000 rpm for 1 hour to remove the debris.

The supernatant was collected and was saturated with 30% ammonium sulphate.

After incubation for 2 hours, this was centrifuged at 6000 rpm for 30 minutes and clear supernatant was collected.

To remove ammonium sulphate, the supernatant was passed on Sephadex G-25 column. Sephadex G-25 column (20 x 1 cm) was packed and equilibrated with phosphate buffered saline. Void volume of the column was determined by passing 1 ml of (5 mg/ml) blue dextran. The void volume was found to be 30 ml. The column was operated at a flow rate of 40 ml/hour. The supernatant obtained from ammonium sulphate precipitation was passed on this Sephadex G-25 column and protein eluting in the void volume was collected and used to determine enzyme activity.

Before reusing, the column was washed extensively with phosphate buffered saline to remove ammonium sulphate.

Human serum and serum from buffalo, goat and rabbit were used as such for enzyme activity estimation.

C. Statistical Evaluation

(a) As the study group is large, observations in this study were statistically evaluated by using 'Z' test. The mean and

standard deviations were calculated with the help of an electronic calculator which provided the above two data directly

$$Z = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

Where n_1, $\bar{X}_1$ and S_1 are respectively the number of items, mean and standard deviation of the values of first series, n_2, $\bar{X}_2$ and S_2 are respectively the number of items, mean and standard deviation of the values of second series.

'p' value was calculated from the probability tables by comparing the value of 'Z'.

(b) Statistical evaluation of the observations was also done using student's 't' test.

$$t = \frac{X_1 - X_2}{S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

The value of S was calculated before hand by the formula:

$$S = \sqrt{\frac{(n_1-1)\,S_1^2 + (n_2-1)\,S_2^2}{n_1 + n_2 - 2}}$$

Where n_1, $\bar{X}_1$ and S_1 are the number of items, mean and standard deviation of the values of first series respectively, n_2, $\bar{X}_2$ and S_2 are the number of items, mean and standard deviation of the values of second series respectively.

The value of 't' thus obtained was compared with the probability tables given by Fischer and Yates and values of 'p' noted. p value less than 0.05 is significant.

RESULTS AND DISCUSSION

III. RESULTS AND DISCUSSION

The level of AST, ALT, AIP, ACP and amylase in different mammalian tissues were determined in appropriate buffer systems. Tissue homogenate was first centrifuged and supernatant was salt-fractionated at 30% ammonium sulphate saturation. The activity was measured in the supernatant after removal of the salt by gel chromatography on Sephadex G-25 column. The specific activity was determined in seven different tissues of buffalo, goat and rabbit; the level of enzymes was also determined in human serum. Table 10 (Figs. 1-3) shows the specific activity of AST in the various tissues of different mammalian species. Strikingly the serum level of AST was found 0.031 $\pm$ 0.001 OD/mg/hr regardless of all the four species listed in Table 10. Similar observations were made on AST activities of skeletal muscle, lung, brain, liver and probably heart. In the latter, the variation in AST activities in different species was about 12% which is not markedly different from the experimental error of 8-10% with which the activity of enzymes could be measured in these studies. The activity in kidney tissue was nearly the same in goat and buffalo but significantly low in rabbit. The discrepancy cannot be ascribed to experimental error, it's significance remained unclear to us. The activity of AST was found to be highest in brain and low in lung and liver. The

TABLE 10: SPECIFIC ACTIVITY OF AST IN DIFFERENT TISSUES OF DIFFERENT SPECIES (MAMMALIAN).

Enzyme	Species	Liver sp.activity OD/mg/hr	Kidney sp.activity OD/mg/hr	Lung sp.activity OD/mg/hr	Heart sp.activity OD/mg/hr	Sk.muscle sp.activity OD/mg/hr	Brain sp.activity OD/mg/hr	Serum sp.activity OD/mg/hr
AST	Buffalo	0.32 (11)	0.98 (33)	0.45 (15)	0.95 (32)	0.89 (30)	1.54 (51)	0.03 (1)
	Goat	0.40 (14)	0.89 (31)	0.38 (13)	0.90 (31)	0.85 (29)	1.82 (63)	0.029 (1)
	Rabbit	0.37 (12)	0.45 (15)	0.43 (14)	1.10 (39)	0.74 (24)	1.70 (55)	0.031 (1)
	Human							0.032

*Values within parentheses indicate relative values taking specific activity in respective serum as unity.

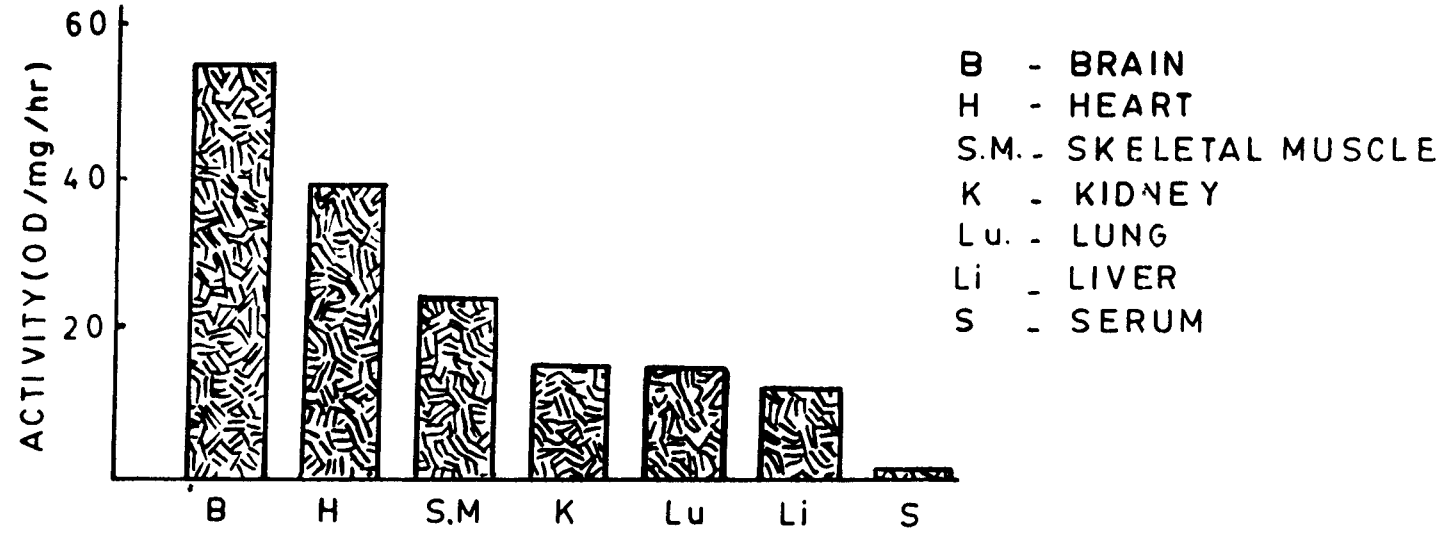


FIG. 1 DIAGRAM SHOWING AST ACTIVITIES IN DIFFERENT TISSUES OF RABBIT TAKING SERUM ACTIVITY AS UNITY

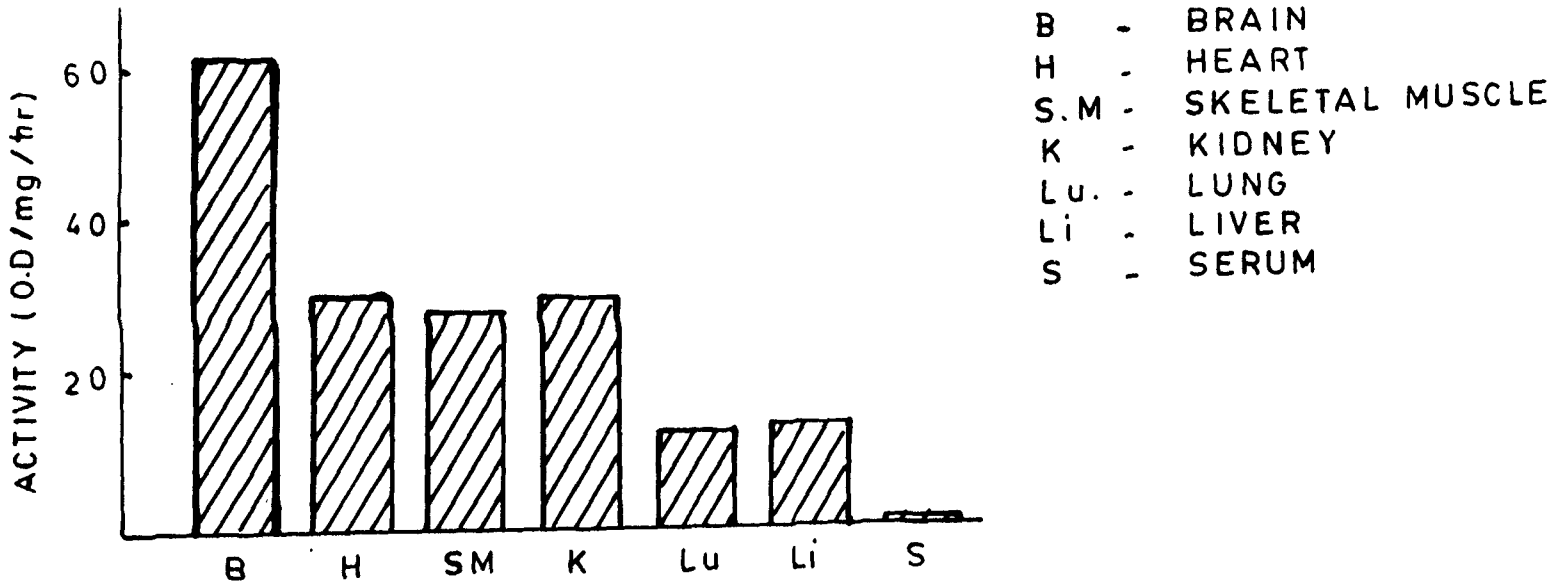


FIG.2 DIAGRAM SHOWING AST ACTIVITIES IN DIFFERENT TISSUES OF GOAT TAKING SERUM ACTIVITY AS UNITY

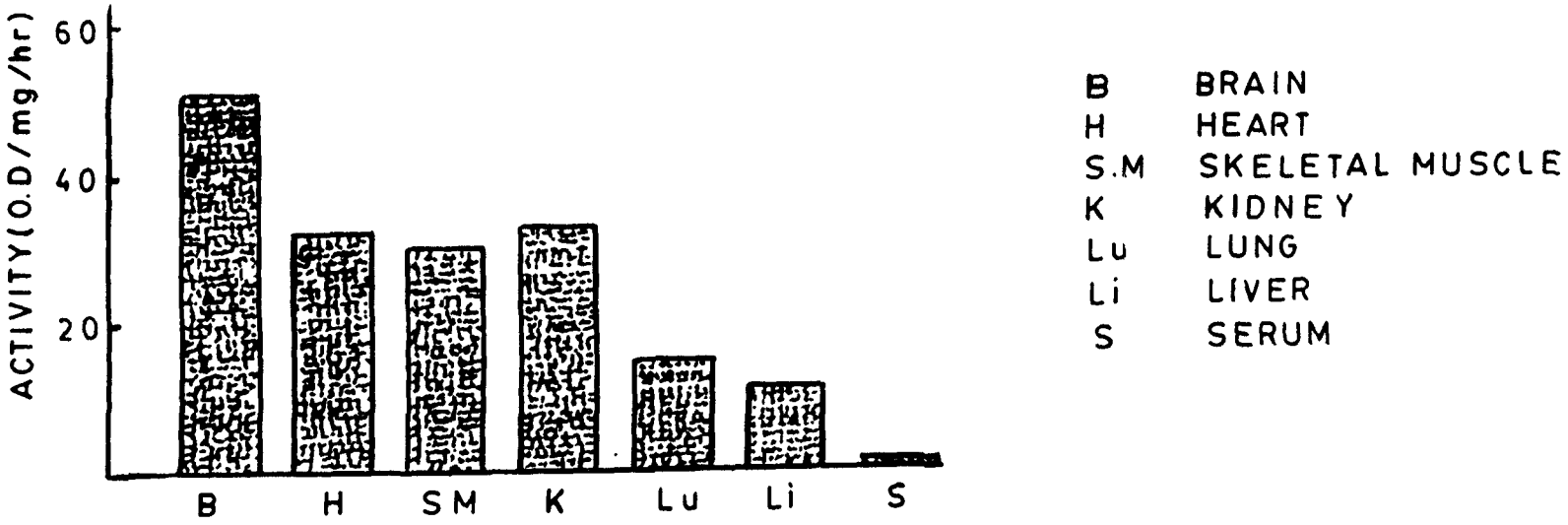


FIG. 3 DIAGRAM SHOWING AST ACTIVITIES IN DIFFERENT TISSUES OF BUFFALO TAKING SERUM ACTIVITY AS UNITY

activities in heart and skeletal muscle were significantly lower than that found in brain tissue but were substantially higher than the values found in lung and liver. These findings regarding tissue distribution correlate well with the findings (Table 6) of other clinical enzymologists on human tissues except that, in present study, the activity in liver was much lower than that reported by others (96,103,104). As the method of preparation of enzyme sample was different in all the reported studies such comparison at best can only be considered futile.

The tissue distribution of ALT activities in buffalo, goat and rabbit is summarised in Table 11 (Figs. 4-6). It can be seen that the species dependent differences for various tissues range from 4-50%. Maximum difference was noted in lung followed by liver. The enzyme levels for buffalo, goat and rabbit kidney were invariant. The difference in enzyme levels of skeletal muscle among the three species was small and hardly significant considering the error of 15% with which the activity could be measured in different independent experiments.

The serum level of ALT was significantly higher in rabbit than those of buffalo and goat serum in which cases the values were similar but not identical to that found in

TABLE 11: SPECIFIC ACTIVITY OF ALT IN DIFFERENT TISSUES OF DIFFERENT SPECIES (MAMMALIAN).

Enzyme	Species	Liver sp.activity OD/mg/hr	Kidney sp.activity OD/mg/hr	Lung sp.activity OD/mg/hr	Heart sp.activity OD/mg/hr	Sk.muscle sp.activity OD/mg/hr	Brain sp.activity OD/mg/hr	Serum sp.activity OD/mg/hr
ALT	Buffalo	0.21 (7.5)	0.75 (27)	0.16 (6)	1.27 (45)	1.54 (55)	1.03 (37)	0.028 (1)
	Goat	0.19 (8)	0.77 (33)	0.16 (7)	2.16 (94)	2.29 (100)	1.94 (84)	0.023 (1)
	Rabbit	0.53 (10)	0.81 (16)	0.44 (9)	1.08 (21)	1.70 (33)	1.52 (30)	0.051 (1)
	Human							0.032

*Values within parentheses indicate relative value taking specific activity in respective serum as unity.

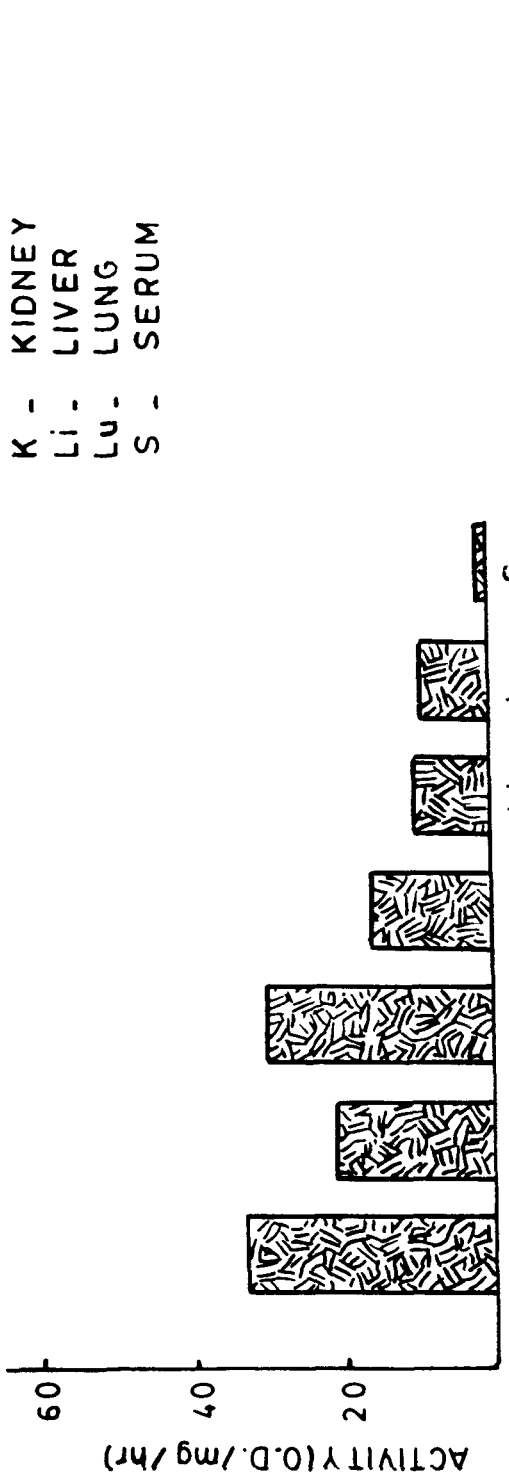


FIG.4 DIAGRAM SHOWING ALT ACTIVITIES IN DIFFERENT TISSUES OF RABBIT TAKING SERUM ACTIVITY AS UNITY

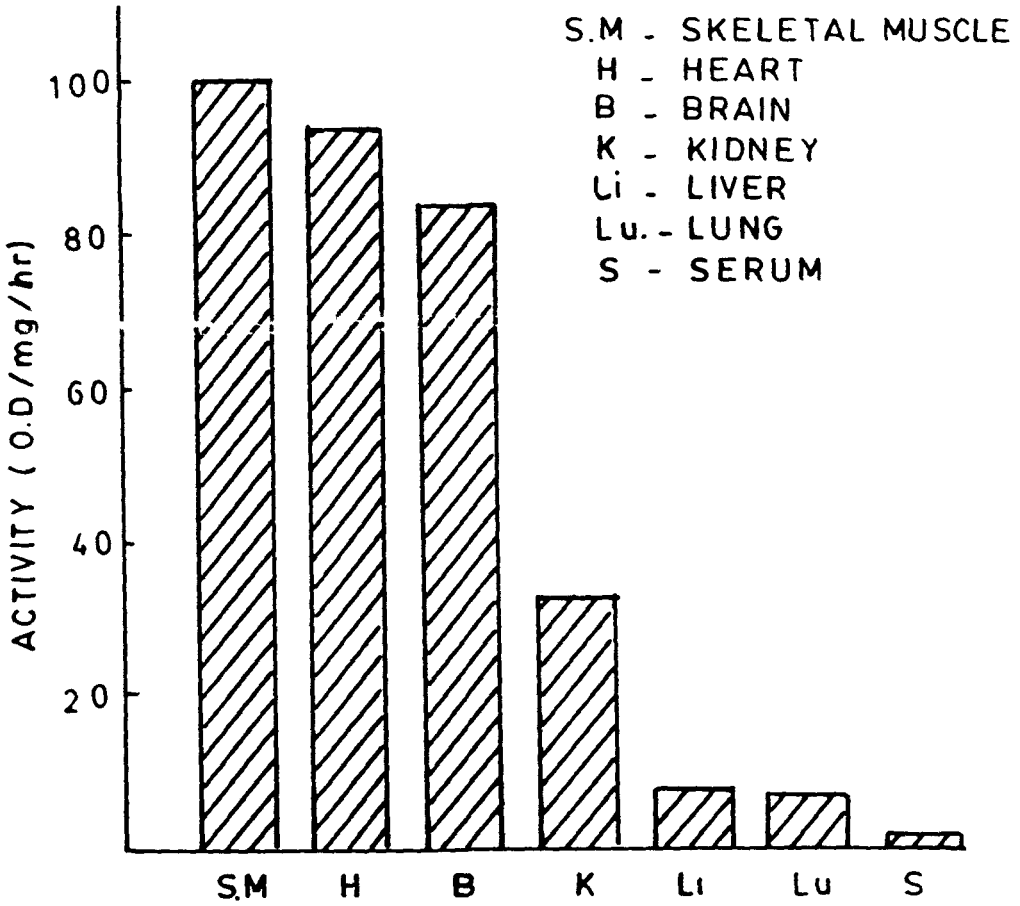
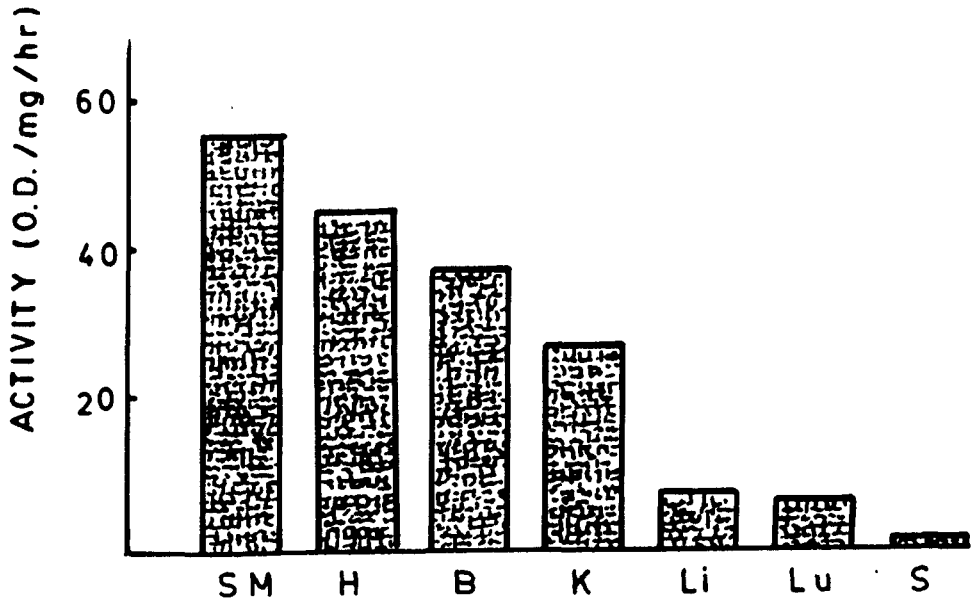


FIG.5 DIAGRAM SHOWING ALT ACTIVITIES IN DIFFERENT TISSUES OF GOAT TAKING SERUM ACTIVITY AS UNITY.



S.M. - SKELETAL MUSCLE
H - HEART
B - BRAIN
K - KIDNEY
Li - LIVER
Lu - LUNG
S - SERUM

FIG. 6 DIAGRAM SHOWING ALT ACTIVITIES IN DIFFERENT TISSUES OF BUFFALO TAKING SERUM ACTIVITY AS UNITY.

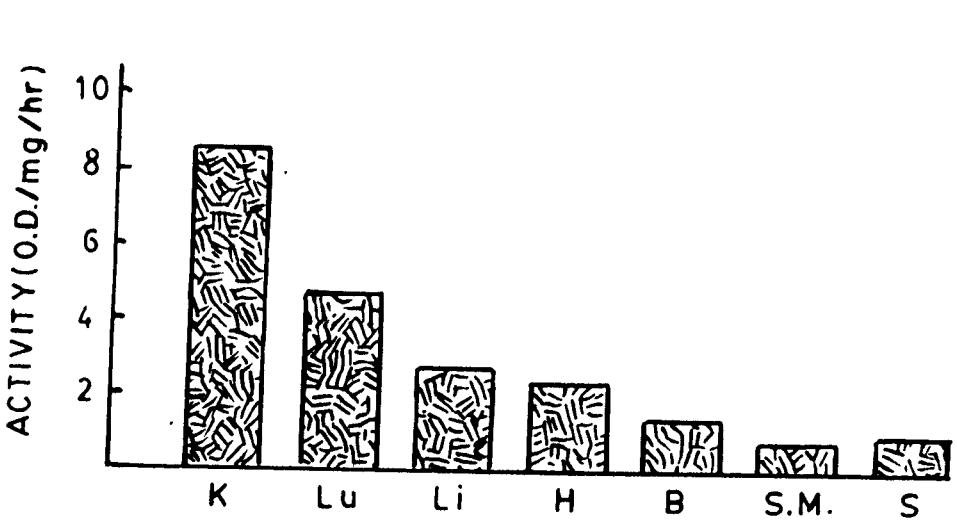
human within the permissible experimental error. For the same species, the level of ALT was found to be markedly higher in skeletal muscle, heart and brain and the values were substantially lower in liver, lung and serum. The ALT level of kidney tissue was in between. Similar pattern was noted for ALT activities in different tissues of buffalo, goat and rabbit. Despite experimental uncertainty these results do suggest that ALT level is higher in skeletal muscle, heart and brain.

Table 12 (Figs. 7-9) shows the level of ALP in different tissues of different mammalian species. In heart and the serum the enzyme concentrations were nearly the same in buffalo, goat and rabbit. Maximum species variation was found in kidney tissue where the average deviation was 52%. In liver, lung, skeletal muscle and brain, the differences among the three species were in the range of 25-29%. Except serum the level of enzyme in other tissues was found to be highest in goat followed by buffalo and rabbit. Tissuewise highest level was measured in kidney followed by lung. The levels in heart, brain and skeletal muscle were substantially lower. It seems that the level of ALP in serum is same regardless of species. Similar conclusions are valid for activity in heart muscle. However, in other tissues the differences in the level of enzyme among three species are small but significant; the lone

TABLE 12: SPECIFIC ACTIVITY OF ALKALINE PHOSPHATASE IN DIFFERENT TISSUES OF DIFFERENT SPECIES (MAMMALIAN).

Enzyme	Species	Liver sp.activity OD/mg/hr	Kidney sp.activity OD/mg/hr	Lung sp.activity OD/mg/hr	Heart sp.activity OD/mg/hr	Sk.muscle sp.activity OD/mg/hr	Brain sp.activity OD/mg/hr	Serum sp.activity OD/mg/hr
Alkaline phosphatase	Buffalo	0.74 (5)	5.14 (34)	2.14 (14)	0.62 (4)	0.19 (1.3)	0.38 (2.5)	0.15 (1)
	Goat	1.17 (6)	10.44 (52)	2.60 (13)	0.73 (3.7)	0.34 (1.7)	0.63 (3)	0.20 (1)
	Rabbit	0.64 (2.7)	2.01 (8.5)	1.11 (4.6)	0.56 (2.3)	0.20 (0.8)	0.34 (1.4)	0.24 (1.0)
	Human							0.20

*Values within parentheses indicate relative value taking specific activity in respective serum as unity.



K . KIDNEY
Lu. LUNG
Li . LIVER
H . HEART
B . BRAIN
S.M. SKELETAL MUSCLE
S SERUM

FIG. 7 DIAGRAM SHOWING ALP ACTIVITIES IN DIFFERENT TISSUES OF RABBIT TAKING SERUM ACTIVITY AS UNITY

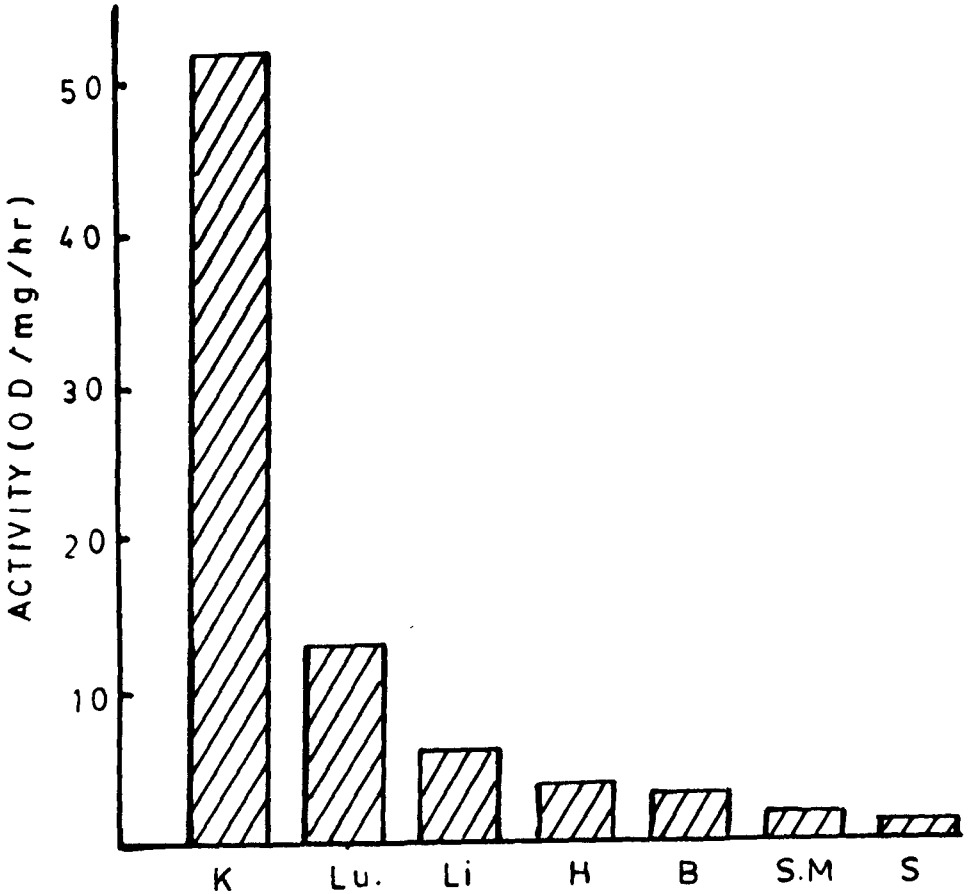
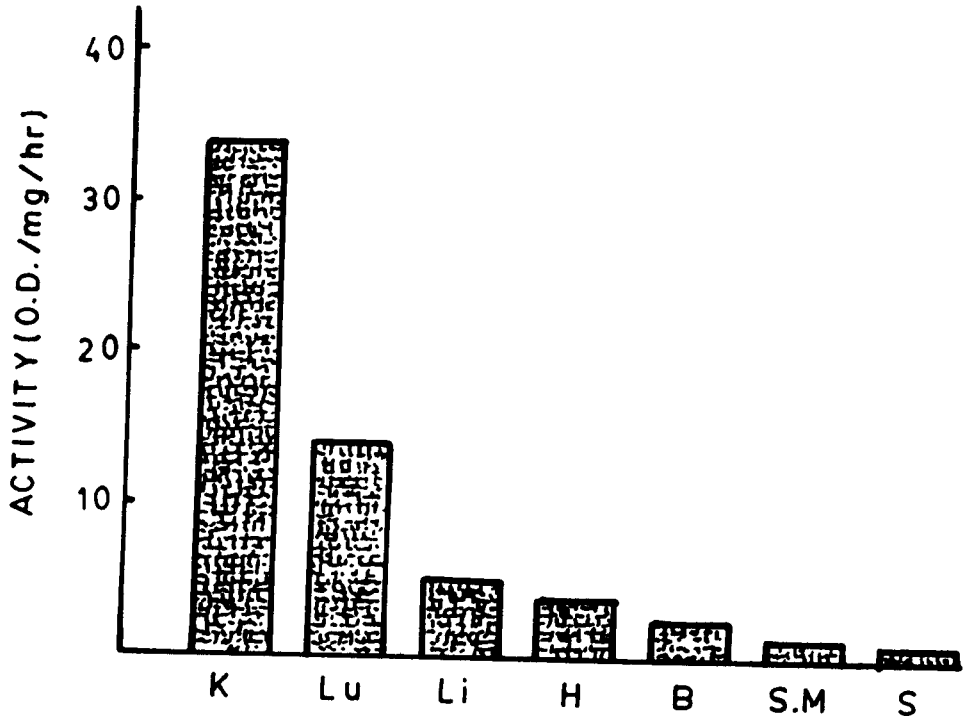


FIG. 8 DIAGRAM SHOWING ALP ACTIVITIES IN DIFFERENT TISSUES OF GOAT TAKING SERUM ACTIVITY AS UNITY.



K - KIDNEY
Lu - LUNG
Li - LIVER
H - HEART
B - BRAIN
S.M - SKELETAL MUSCLE
S - SERUM

FIG.9 DIAGRAM SHOWING ALP ACTIVITIES IN DIFFERENT TISSUES OF BUFFALO TAKING SERUM ACTIVITY AS UNITY

TABLE 13: SPECIFIC ACTIVITY OF ACID PHOSPHATASE IN DIFFERENT TISSUES OF DIFFERENT SPECIES (MAMMALIAN).

Enzyme	Species	Liver sp.activity OD/mg/hr	Kidney sp.activity OD/mg/hr	Lung sp.activity OD/mg/hr	Heart sp.activity OD/mg/hr	Sk.muscle sp.activity OD/mg/hr	Brain sp.activity OD/mg/hr	Serum sp.activity OD/mg/hr
Acid phosphatase	Buffalo	0.27 (135)	0.41 (205)	0.38 (190)	0.25 (125)	0.25 (125)	0.56 (280)	0.002 (1)
	Goat	0.43 (215)	0.76 (380)	0.56 (280)	0.26 (130)	0.22 (110)	0.86 (430)	0.002 (1)
	Rabbit	0.21 (16)	0.28 (21.5)	0.23 (18)	0.21 (16)	0.20 (15)	0.53 (41)	0.013 (1)
	Human							0.002

*Values within parentheses indicate relative value taking specific activity in respective serum as unity.

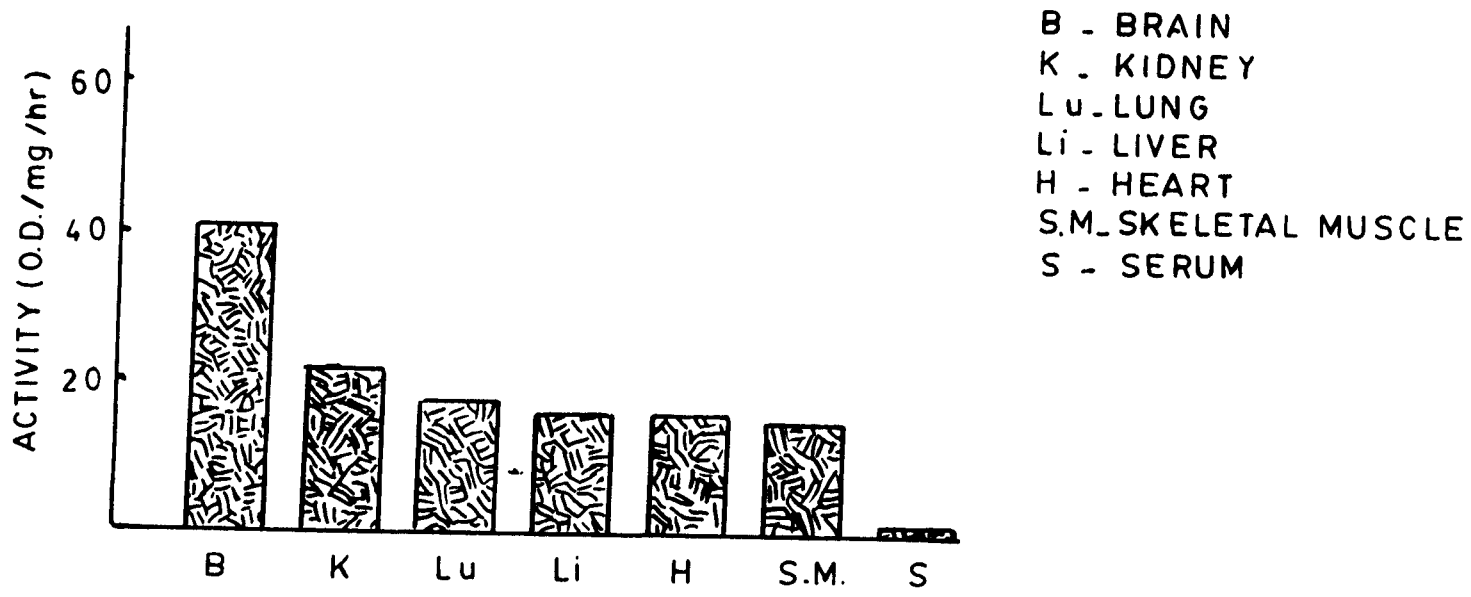


FIG. 10 DIAGRAM SHOWING ACP ACTIVITIES IN DIFFERENT TISSUES OF RABBIT TAKING SERUM ACTIVITY AS UNITY

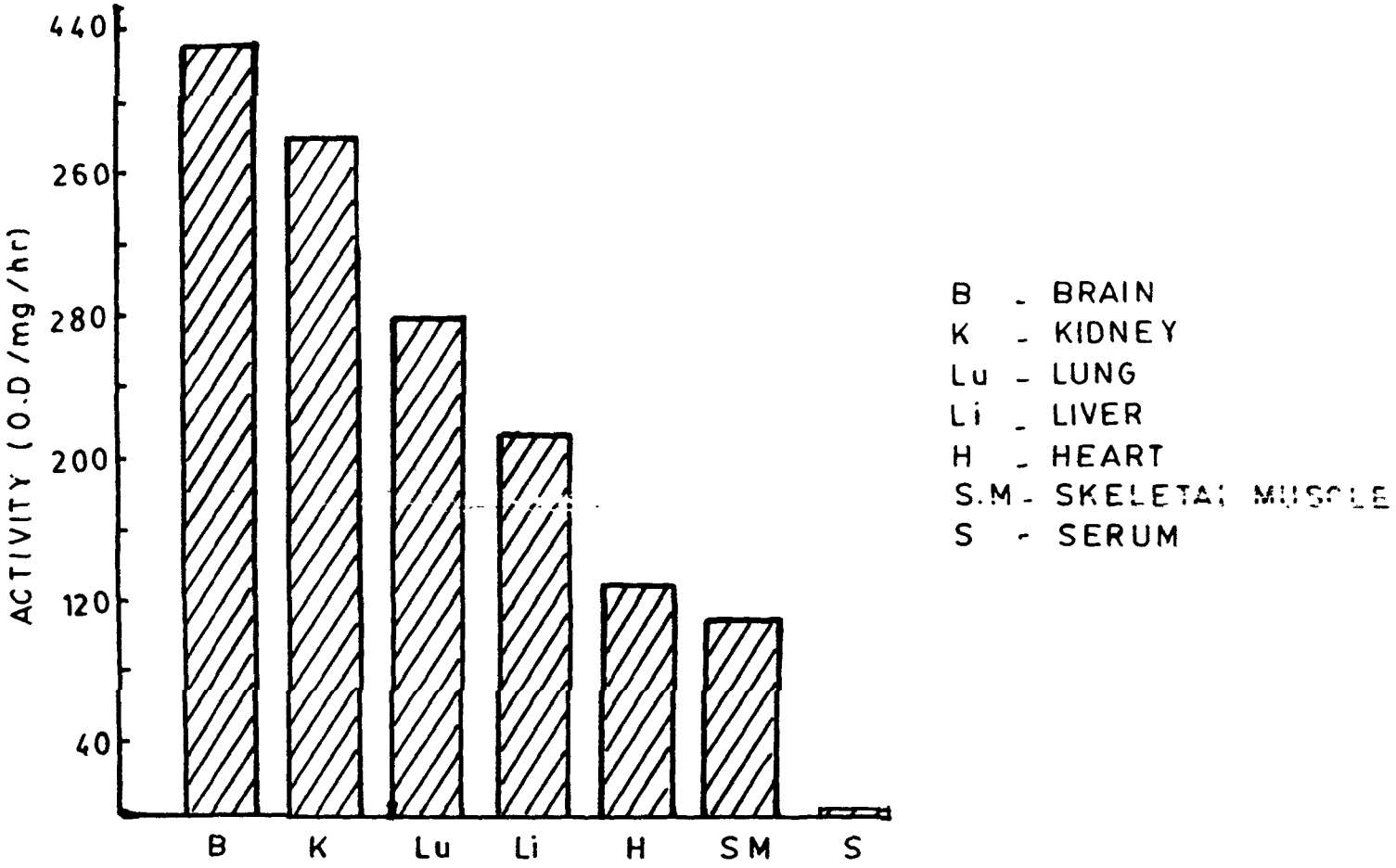


FIG.11 DIAGRAM SHOWING ACP ACTIVITIES IN DIFFERENT TISSUES OF GOAT TAKING SERUM ACTIVITY AS UNITY

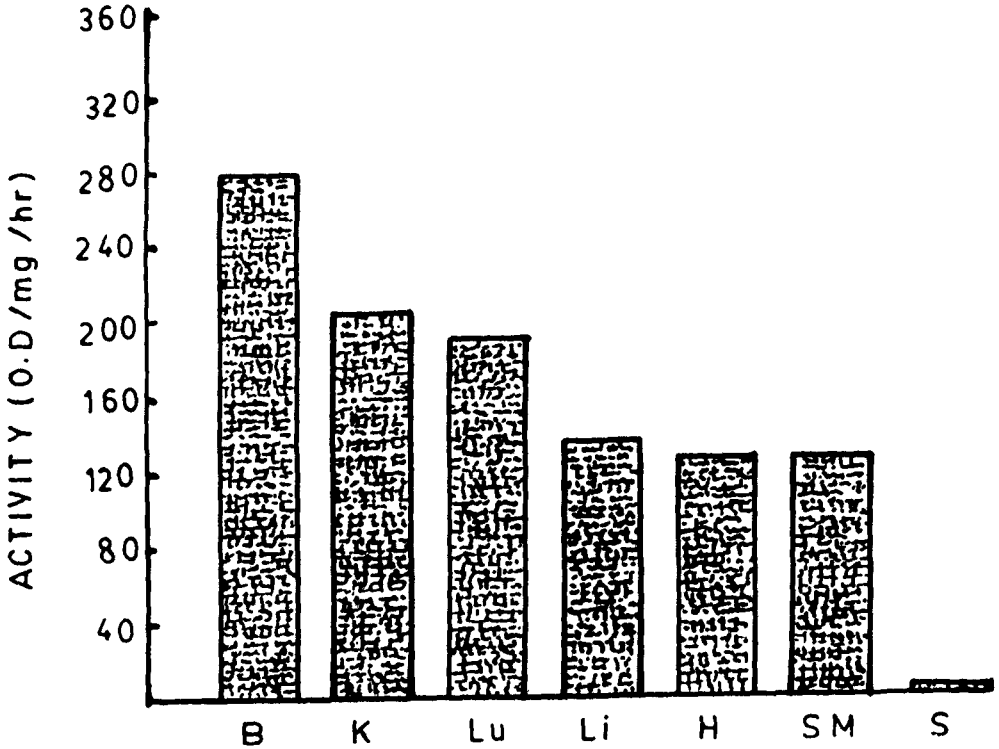


FIG.12 DIAGRAM SHOWING ACP ACTIVITIES IN DIFFERENT TISSUES OF BUFFALO TAKING SERUM ACTIVITY AS UNITY

exception appears to be kidney where the variation among the three species is substantial.

Tissue distribution of ACP is summarised in Table 13 and is graphically shown in Figs. 10-12. Analysis of the results shows that the levels of ACP in heart and skeletal muscle were the same for all the species namely buffalo, goat and rabbit. In other tissues the variation among the three species were between 22-38%. Maximum deviation was noted in case of kidney. The serum level of enzyme was found to be invariably low in human, rabbit, goat and buffalo in comparison to other body tissues. The level of ACP was significantly higher in brain tissue of all the three species followed by kidney and lung.

The levels of amylase in different tissues of buffalo, goat and rabbit are summarised in Table 14 and tissue distribution for enzyme of each species is depicted in Figs. 13-15.

The serum levels of amylase in human, rabbit, goat and buffalo were found to be same within experimental error. The amylase activity of lung, brain, heart and serum were almost similar. The enzyme levels in liver and kidney were substantially higher regardless of the species. The highest level was found in the liver followed by kidney. The lowest level was found in the skeletal muscle.

TABLE 14: SPECIFIC ACTIVITY OF AMYLASE IN DIFFERENT TISSUES OF DIFFERENT SPECIES (MAMMALIAN)

Enzyme	Species	Liver sp.activity OD/mg/hr	Kidney sp.activity OD/mg/hr	Lung sp.activity OD/mg/hr	Heart sp.activity OD/mg/hr	Sk.muscle sp.activity OD/mg/hr	Brain sp.activity OD/mg/hr	Serum sp.activity OD/mg/hr
Amylase	Buffalo	0.99 (3.5)	1.11 (4)	0.30 (1.1)	0.48 (1.7)	0.33 (1.2)	0.38 (1.4)	0.28 (1.0)
	Goat	0.37 (1.5)	0.24 (1.0)	0.23 (0.9)	0.26 (1.0)	0.18 (0.7)	0.15 (0.6)	0.25 (1.0)
	Rabbit	0.36 (1.1)	0.29 (0.9)	0.34 (1.0)	0.26 (0.8)	0.14 (0.4)	0.24 (0.7)	0.34 (1.0)
	Human							0.28

*Values within parentheses indicate relative value taking specific activity in respective serum as unity.

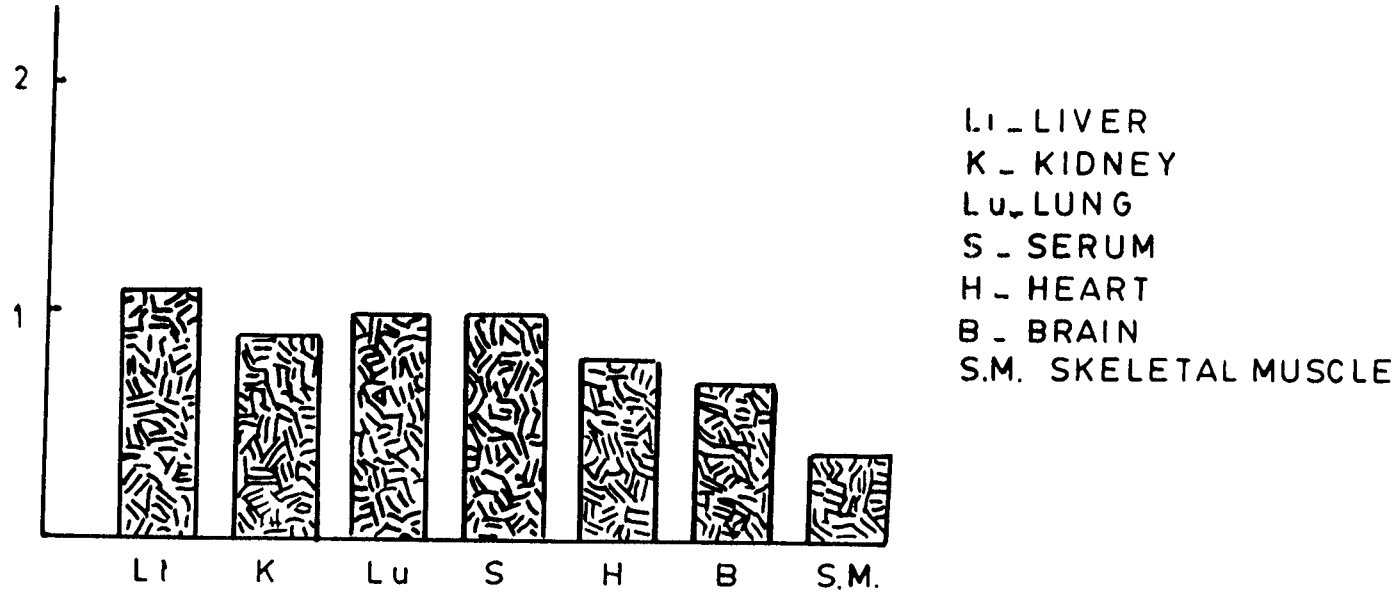


FIG 13 DIAGRAM SHOWING AMYLASE ACTIVITIES IN DIFFERENT TISSUES OF RABBIT TAKING SERUM ACTIVITY AS UNITY.

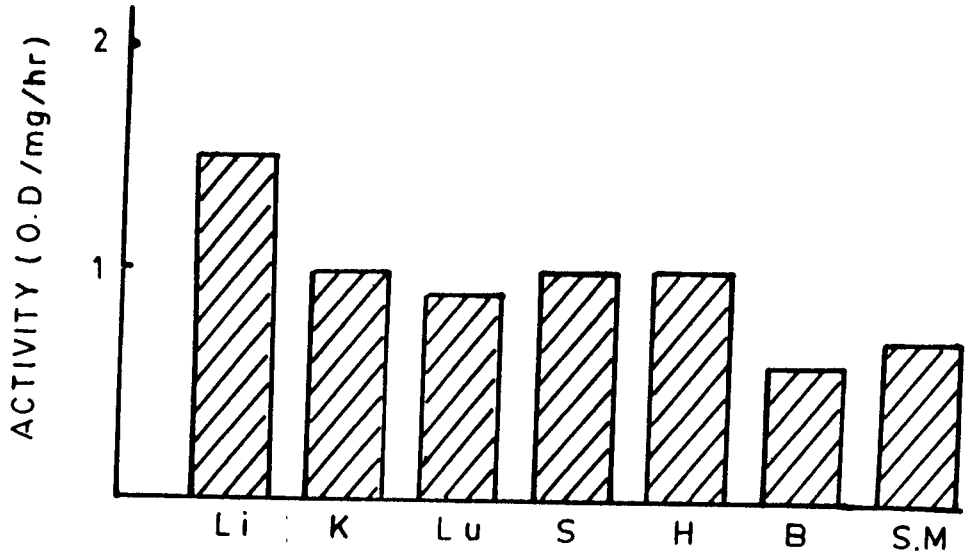


FIG 14 DIAGRAM SHOWING AMYLASE ACTIVITIES IN DIFFERENT TISSUES OF GOAT TAKING SERUM ACTIVITY AS UNITY

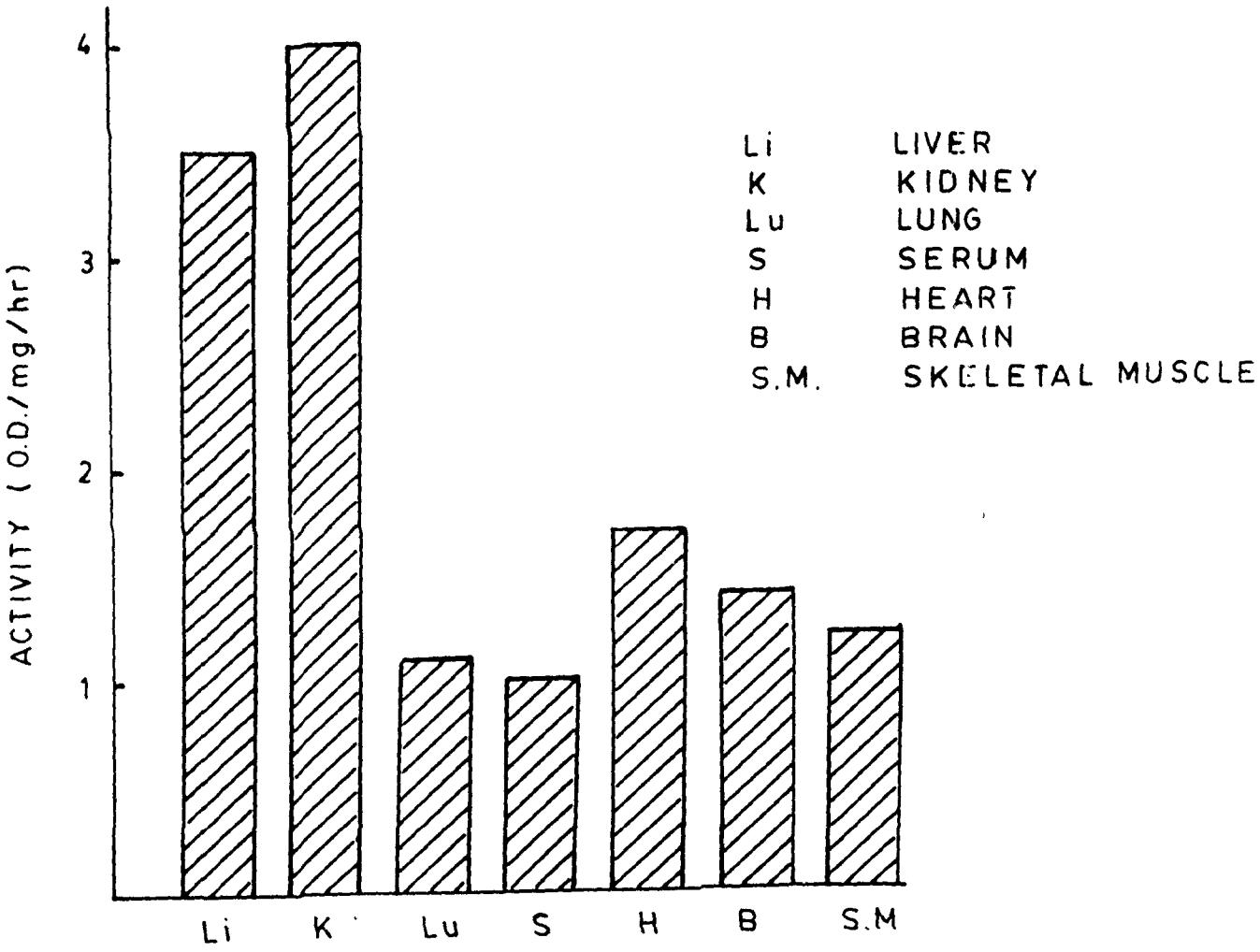


FIG. 15 DIAGRAM SHOWING AMYLASE ACTIVITIES IN DIFFERENT TISSUES OF GOAT TAKING SERUM ACTIVITY AS UNITY.

TABLE 15 : AGE AND SEX DISTRIBUTION OF STUDY GROUP.

S.No.	Age in years	Total cases		Males		Females	
		Total number	Percentage	Total number	Percentage	Total number	Percentage
1.	0 - 10	13	6.5	10	76.9	3	23.1
2.	10 - 20	37	18.5	23	62.2	14	37.8
3.	20 - 30	43	21.5	19	44.2	24	55.8
4.	30 - 40	36	18.0	23	63.9	13	36.1
5.	40 - 50	31	15.5	21	67.7	10	32.3
6.	50 - 60	19	9.5	16	84.2	3	15.8
7.	60 - 70	21	10.5	17	81.0	4	19.0
	Total	200	100.0	129	64.5	71	35.5

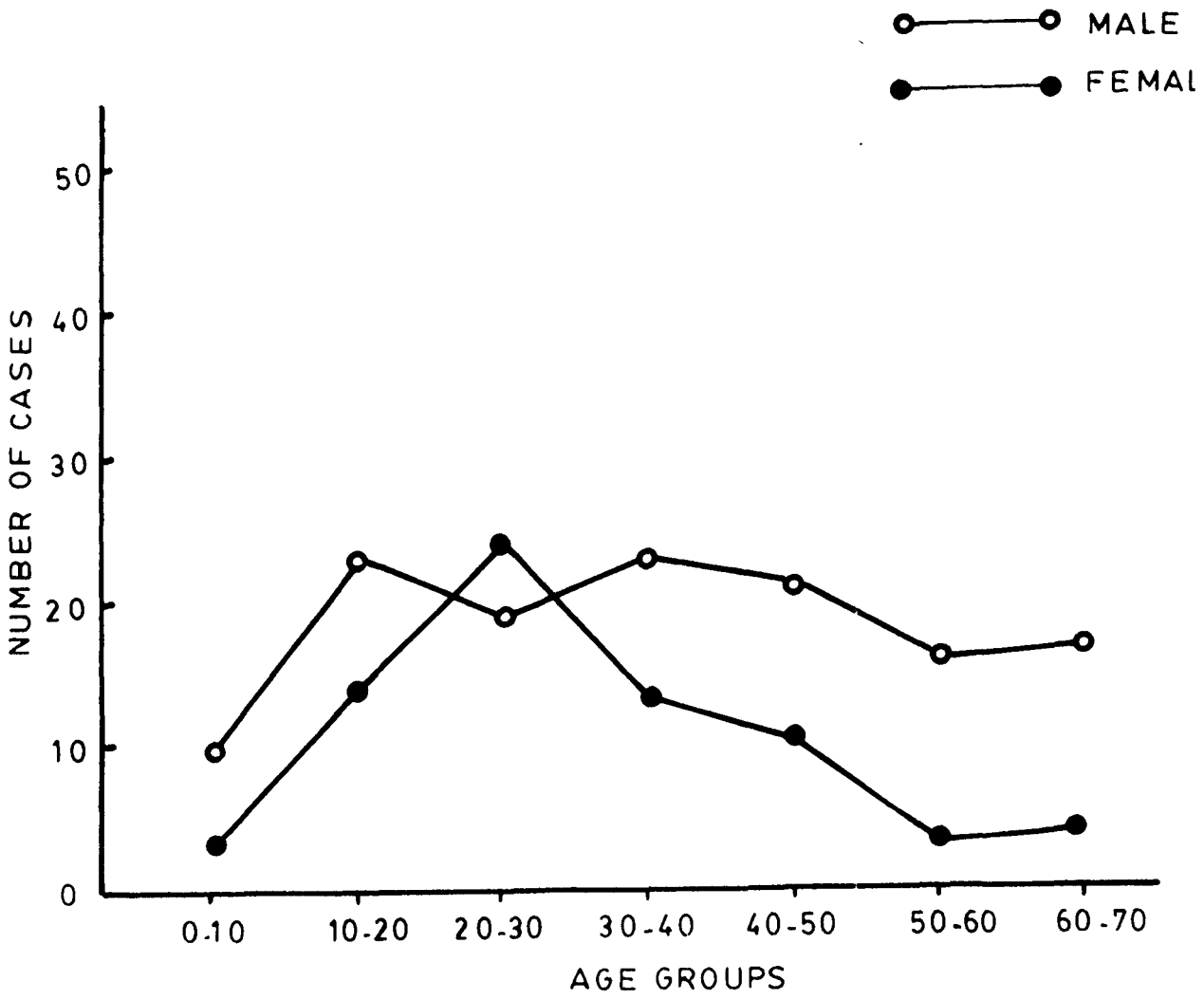


FIG.16 GRAPH SHOWING DISTRIBUTION OF MALE AND FEMALE CASES IN DIFFERENT AGE GROUPS

TABLE 16: COMPARISON OF NORMAL SERUM ENZYME PROFILES OF ALIGARH POPULATION WITH LITERATURE VALUE (12).

S.No.	Enzyme	Activity (Mean ± 2 S.D.)	
		This study	Literature value(12)
1.	AST (I.U./L)	13.8 ± 9.2	12.5 ± 7.5
2.	ALT (IU/L)	9.3 ± 5.9	10.0 ± 5.0
3.	Alkaline phosphatase (KA/dl)	8.6 ± 5.0	8.0 ± 5.0
4.	Acid phosphatase (KA/dl)	1.5 ± 1.1	2.2. ± 1.3
5.	Amylase (S.U/dl)	180.8 ± 32.5	130.0 ± 70.0

After investigating the distribution of AST, ALT, ALP, ACP and amylase in various tissues of three mammalian systems, we set out to investigate the normal level of these clinically important enzymes in two hundred blood samples received from clinically healthy persons of Aligarh. The result of the levels of all the five enzymes are tabulated in Appendix I. Statistical analysis of the results was done as described in the experimental section. The results of this analysis are listed in Tables 15-23.

The Table 15 shows the age and sex distribution of the study group. The distribution is also shown graphically in Fig. 16. The maximum number of cases (21.5%) were in the age group of 20-30 years. However, lowest number (6.5%) were in the age group of 0-10 years.

In this study there were more male cases (64.5%) as compared to female cases (35.5%).

It can be seen from Table 16 that the normal values of serum enzymes found in this study are in good agreement with those reported in literature (105-107). However, the normal amylase level found in this study on Aligarh population is significantly on higher range.

Analysis of the serum enzyme level was also done according to sex. The serum enzyme profiles in different sex

TABLE 17: SERUM ENZYME PROFILES IN DIFFERENT SEX GROUPS.

S.No. Cases	Total No. of cases	Percentage	AST (IU/L)	ALT (IU/L)	Alk.phosph. (KA/dl)	Acid phosph. (KA/dl)	Amylase (SV/dl)
			Mean±2 S.D.	Mean±2 S.D.	Mean±2 S.D.	Mean±2 S.D.	Mean±2 S.D.
1. Male	129	64.5	14.3±8.8	9.5±5.8	8.5±5.0	1.6±1.3	180.9±32.1
2. Female	71	35.5	12.9±9.6	9.2±6.0	8.7± 5.0	1.4±0.6	180.7±31.9
Total	200	100.0	13.8±9.2	9.3±5.9	8.6±5.0	1.5±1.1	180.8±32.5
1:2 p value			$p < 0.05$	$p > 0.05$	$p > 0.05$	$p < 0.01$	$p > 0.05$

*p value is obtained by 'Z' test.

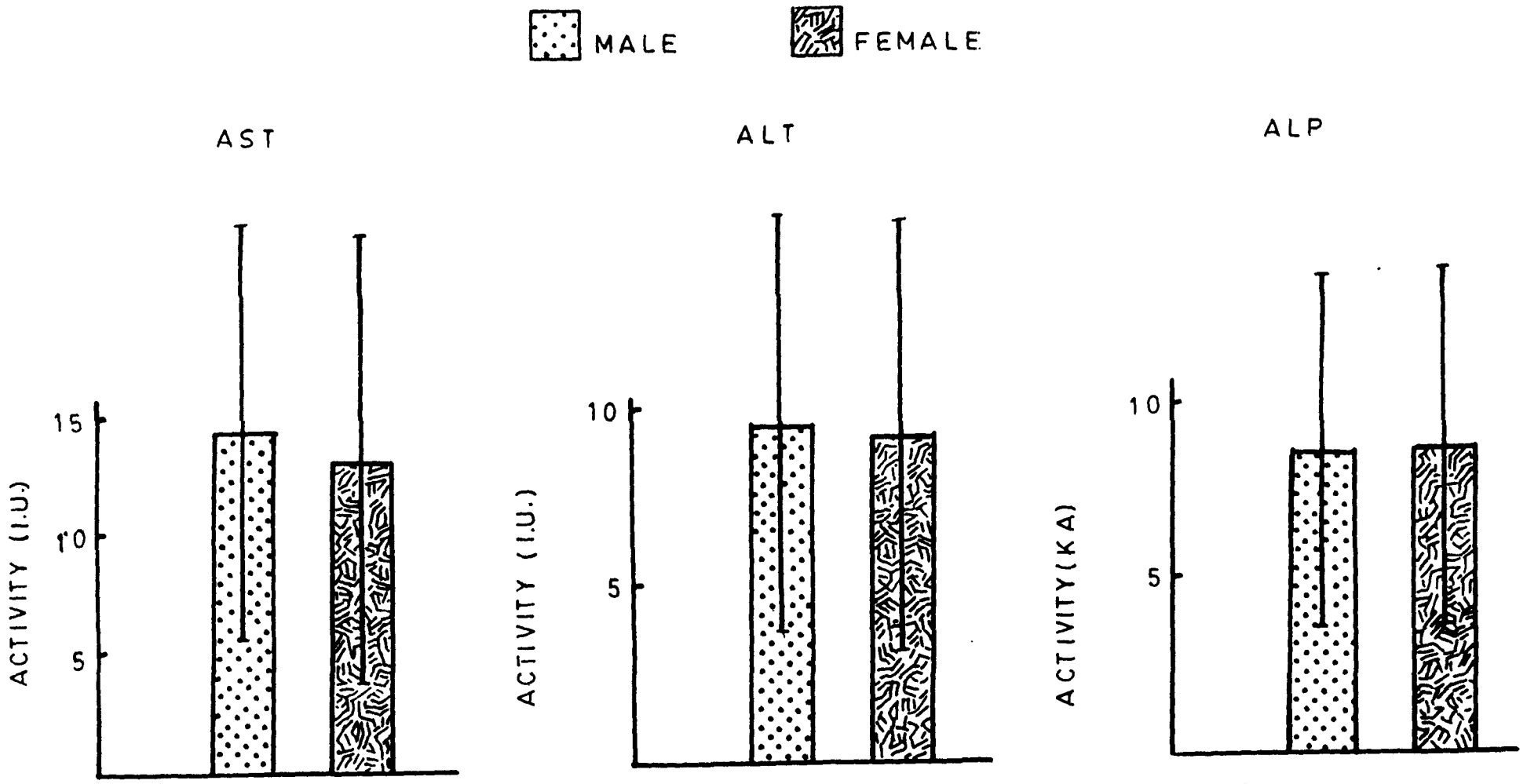


FIG. 17 DIAGRAM SHOWING SERUM ENGYME PROFILES IN DIFFERENT SEX GROUPS

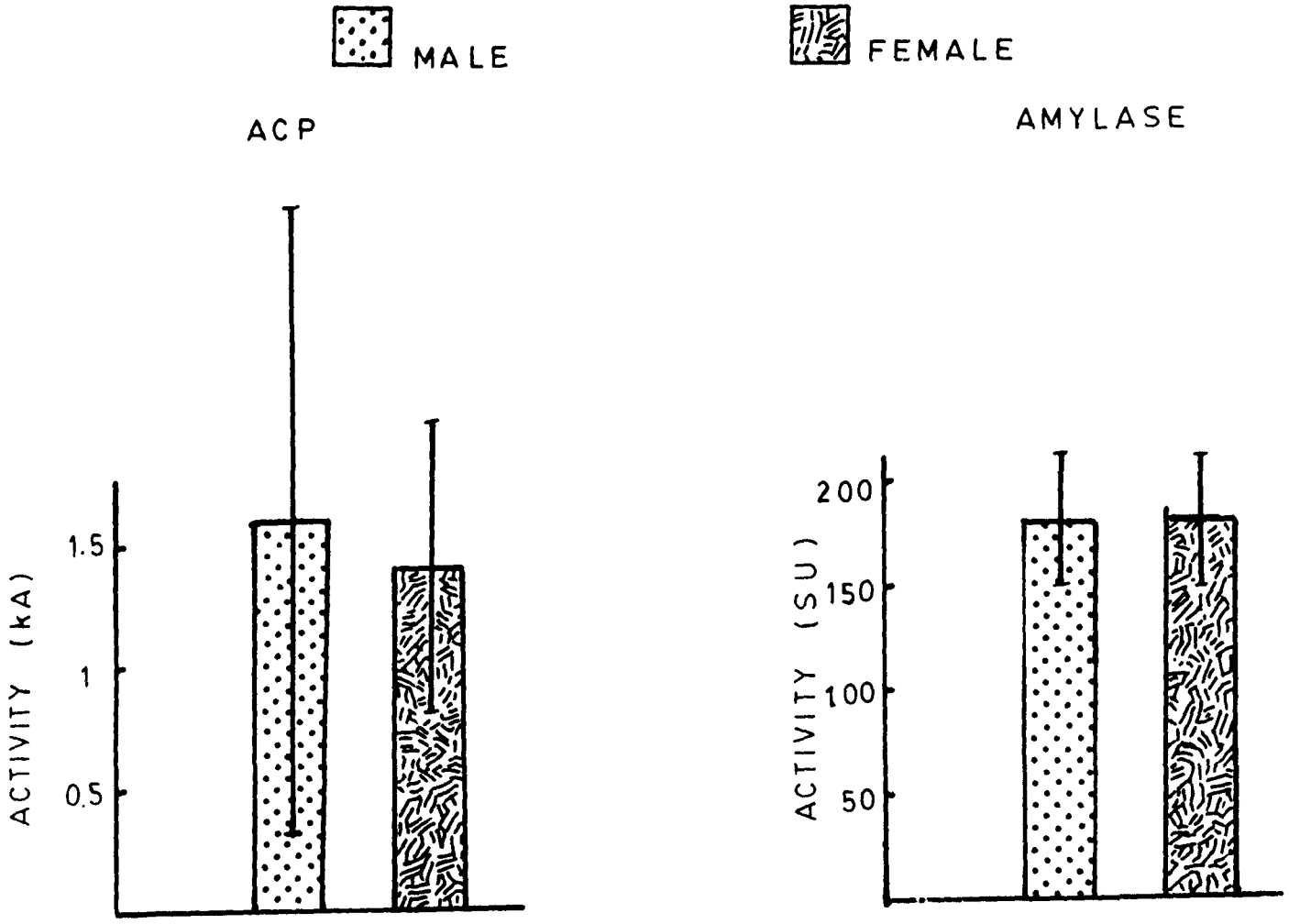


FIG. 18 DIAGRAM SHOWING SERUM ENZYME PROFILES IN DIFFERENT SEX GROUPS.

groups are listed in Table 17 (Figs. 17,18). Statistically significant differences were obtained in AST ($p < 0.05$) and acid phosphatase values ($p < 0.01$) between males and females with higher values in male. The sex dependent differences in the activities of the rest of the enzymes seem to be statistically insignificant ($p > 0.05$). Generally males tend to exhibit somewhat higher levels of alkaline phosphatase than do women. This may be related to differences in skeletal mass, but it is also related to greater physical activity of the male which serves as a stimulus for an increase in bone formation (108,109). Healthy males also show slightly higher transaminase levels than females (33).

Table 18 (Figs. 19,20) depicts the serum enzyme profiles in children (0-12 years) and adult (12-70 years) age groups. Statistically significant higher values of acid phosphatase ($p < 0.001$) were found in children-age-groups in comparison to the adults. For other enzymes, the difference between children and adult age groups is not statistically significant ($p > 0.05$). The level of both acid and alkaline phosphatases is higher in growing children than in adult. This is due to the increased bone growth at this age (109,110-112). Transaminase level is also higher in children-age-group (111).

Table 19 shows the serum AST level in different age and sex groups. Statistically significant differences in the

TABLE 18: SERUM ENZYME PROFILES IN CHILDREN AND ADULT AGE GROUPS.

S.No.	Age group (years)	Total No. of cases	Percentage	AST (IU/L)	ALT (IU/L)	Alk.phosph. (KA/dl)	Acid phosph. (KA/dl)	Amylase (SW/dl)
				Mean±2 S.D.	Mean±2 S.D.	Mean±2 S.D.	Mean±2 S.D.	Mean±2 S.D.
1.	0 - 12	15	7.5	14.4±7.8	9.5±6.0	8.8±4.3	2.0±0.6	179.3±29.1
2.	12 - 70	185	92.5	13.6±9.4	9.2±5.6	8.5±5.2	1.4±0.9	180.8±32.2
	Total	200	100.0	13.8±9.2	9.3±5.9	8.6±5.0	1.5±1.1	180.8±32.5
1:2	p value			$p > 0.05$	$p > 0.05$	$p > 0.05$	$p < 0.001$	$p > 0.05$

*p value is obtained by 'Z' test.

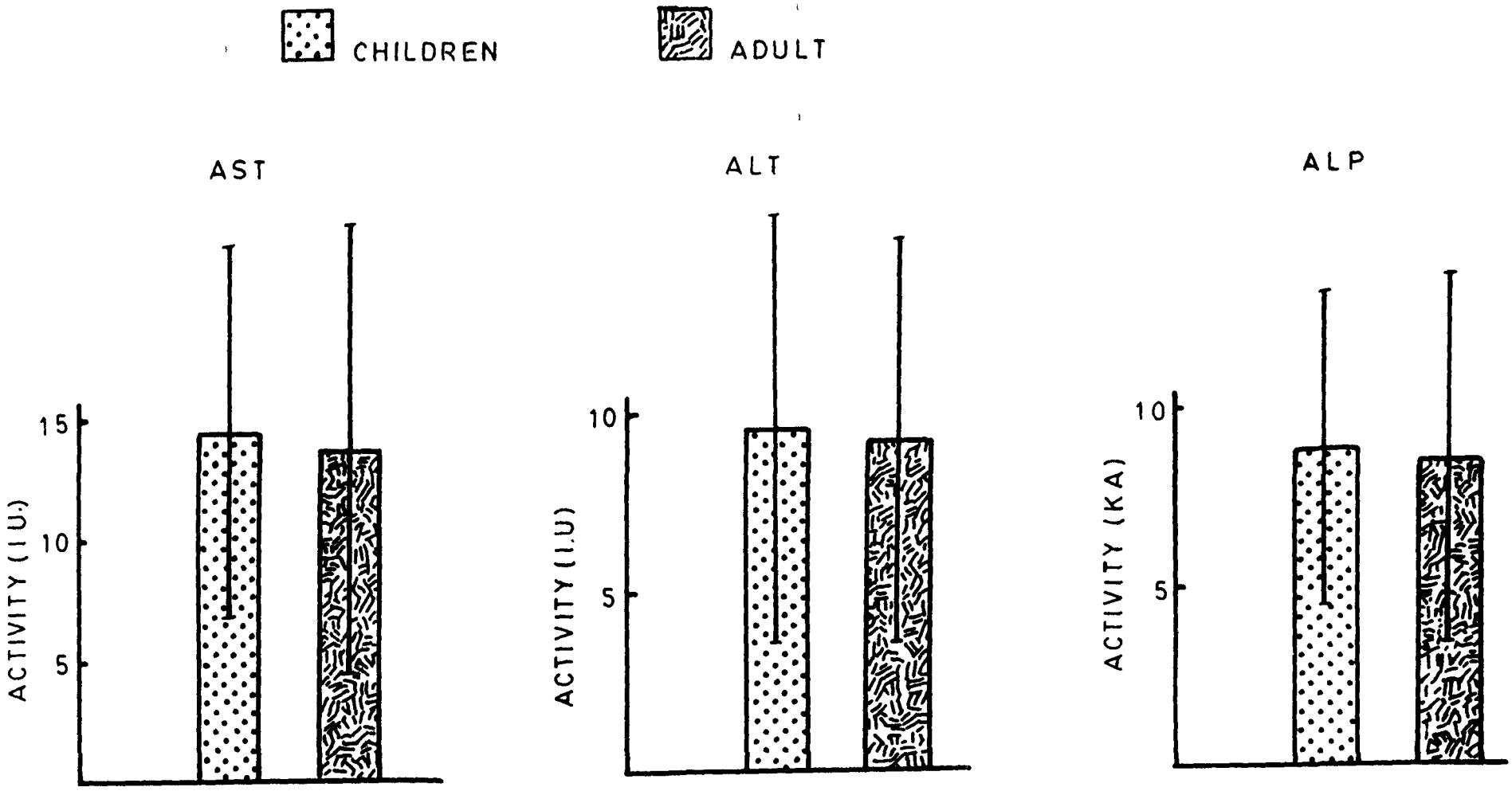


FIG. 19 DIAGRAM SHOWING SERUM ENGYME PROFILES IN CHILDREN AND ADULT AGE GROUPS

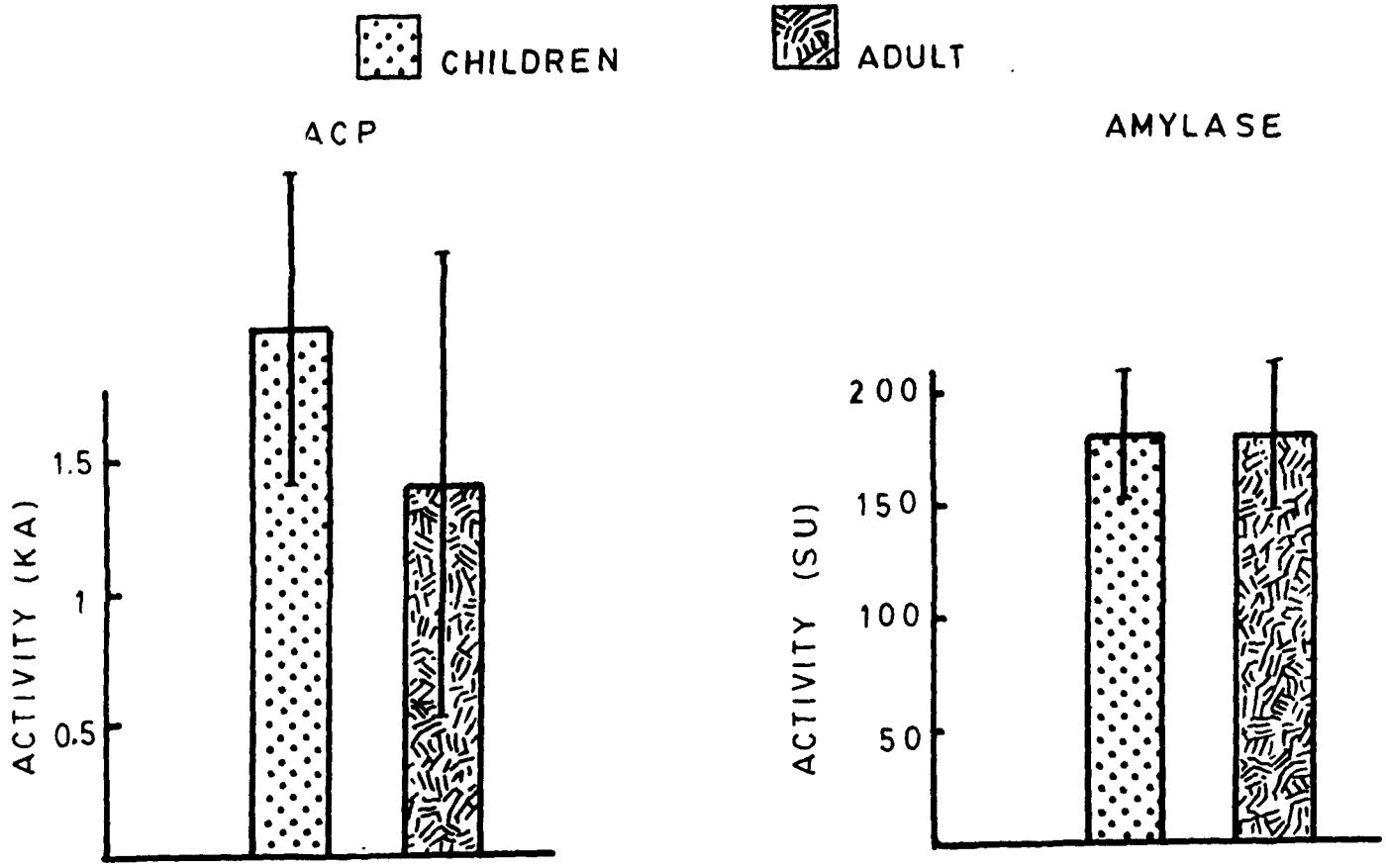


FIG. 20 DIAGRAM SHOWING SERUM ENGYME PROFILES IN CHILDREN AND ADULT AGE GROUPS

TABLE 19: SERUM AST LEVELS IN DIFFERENT AGE AND SEX GROUPS.

S.No.	Age group	Total No. of cases		AST (mean ± 2 S.D.) IU/L			P value* M:F
		Male	Female	Male (M)	Female (F)	Total cases	
1.	0 - 10	10	3	13.9 ± 8.2	13.7 ± 5.7	13.8 ± 7.7	$p > 0.05$
2.	10 - 20	23	14	14.0 ± 8.7	10.9 ± 8.7	12.8 ± 9.1	$p < 0.05$
3.	20 - 30	19	24	12.4 ± 9.8	13.7 ± 9.8	13.1 ± 9.8	$p > 0.05$
4.	30 - 40	23	13	14.5 ± 8.7	12.5 ± 10.2	13.8 ± 9.5	$p > 0.05$
5.	40 - 50	21	10	15.0 ± 8.0	14.2 ± 9.3	14.7 ± 8.5	$p > 0.05$
6.	50 - 60	16	3	15.5 ± 6.7	10.0 ± 4.9	14.6 ± 7.6	$p < 0.05$
7.	60 - 70	17	4	15.1 ± 8.3	14.3 ± 7.9	15.0 ± 8.2	$p > 0.05$
	Total	129	71	14.3 ± 8.8	12.9 ± 9.6	13.8 ± 9.2	-

*P value is obtained by 't' test.

TABLE 20: SERUM ALT LEVELS IN DIFFERENT AGE AND SEX GROUPS.

S.No.	Age group (years)	Total No. of cases		ALT (Mean ± 2 SD) (IU/L)			P value* M:F
		Male	Female	Male (M)	Female (F)	Total cases	
1.	0 - 10	10	3	9.2 ± 5.7	10.7 ± 4.7	9.5 ± 5.6	$p > 0.05$
2.	10 - 20	23	14	10.2 ± 6.8	8.7 ± 5.2	9.6 ± 6.4	$p > 0.05$
3.	20 - 30	19	24	9.3 ± 6.5	9.2 ± 6.1	9.2 ± 6.3	$p > 0.05$
4.	30 - 40	23	13	9.3 ± 5.5	9.6 ± 6.3	9.4 ± 5.8	$p > 0.05$
5.	40 - 50	21	10	9.6 ± 4.7	9.4 ± 5.9	9.5 ± 5.1	$p > 0.05$
6.	50 - 60	16	3	9.9 ± 5.8	7.3 ± 2.5	9.5 ± 5.7	$p > 0.05$
7.	60 - 70	17	4	8.4 ± 3.7	9.8 ± 7.8	8.7 ± 4.9	$p > 0.05$
	Total	129	71	9.5 ± 5.8	9.2 ± 6.0	9.3 ± 5.9	-

*p value is obtained by 't' test.

TABLE 21: SERUM ALKALINE PHOSPHATASE LEVELS IN DIFFERENT AGE AND SEX GROUPS.

S.No.	Age group (years)	Total No. of cases		Alkaline phosphatase (mean±2SD)(KA/dl.)			p value* M:F
		Male	Female	Male (M)	Female (F)	Total cases	
1.	0 - 10	10	3	8.7 ± 4.4	8.5 ± 4.6	8.7 ± 4.5	$p > 0.05$
2.	10 - 20	23	14	8.7 ± 2.0	7.5 ± 4.8	8.2 ± 4.5	$p < 0.05$
3.	20 - 30	19	24	8.2 ± 4.8	9.4 ± 4.1	8.9 ± 4.6	$p > 0.05$
4.	30 - 40	23	13	8.8 ± 5.7	8.5 ± 5.0	8.7 ± 5.5	$p > 0.05$
5.	40 - 50	21	10	8.7 ± 4.6	8.2 ± 5.3	8.5 ± 4.8	$p > 0.05$
6.	50 - 60	16	3	8.0 ± 4.9	9.0 ± 4.3	8.3 ± 4.9	$p > 0.05$
7.	60 - 70	17	4	8.5 ± 5.7	10.4 ± 5.2	8.8 ± 5.8	$p > 0.05$
	Total	129	71	8.5 ± 5.0	8.7 ± 5.0	8.6 ± 5.0	-

*p value is obtained by 't' test.

TABLE 22 : SERUM ACID PHOSPHATASE LEVELS IN DIFFERENT AGE AND SEX GROUPS.

S.No.	Age group	Total No. of cases		Acid phosphatase (Mean ± 2 SD) (KA/dl)			P value* M:F
		Male	Female	Male (M)	Female (F)	Total cases	
1.	0 - 10	10	3	2.1 ± 0.7	1.9 ± 0.2	2.0 ± 0.6	$p > 0.05$
2.	10 - 20	23	14	1.5 ± 0.9	1.2 ± 0.6	1.4 ± 0.8	$p < 0.05$
3.	20 - 30	19	24	1.3 ± 0.5	1.4 ± 0.6	1.4 ± 0.5	$p > 0.05$
4.	30 - 40	23	13	1.5 ± 1.4	1.5 ± 0.6	1.5 ± 1.2	$p > 0.05$
5.	40 - 50	21	10	1.9 ± 1.5	1.3 ± 0.4	1.7 ± 1.4	$p < 0.05$
6.	50 - 60	16	3	1.4 ± 0.8	1.2 ± 0.2	1.4 ± 0.7	$p > 0.05$
7.	60 - 70	17	4	1.6 ± 1.6	1.4 ± 0.9	1.6 ± 1.5	$p > 0.05$
	Total	129	71	1.6 ± 1.3	1.4 ± 0.6	1.5 ± 1.1	-

*p value is obtained by 't' test.

TABLE 23: SERUM AMYLASE LEVELS IN DIFFERENT AGE AND SEX GROUPS.

S.No.	Age group (years)	Total No. of cases		Amylase (Mean ± 2 SD) (SU./dl)			p value* M:F
		Male	Female	Male (M)	Female (F)	Total cases	
1.	0 - 10	10	3	182.8 ± 28.7	177.7 ± 29.6	181.2 ± 29.1	$p > 0.05$
2.	10 - 20	23	14	181.5 ± 24.3	179.8 ± 24.6	180.9 ± 24.5	$p > 0.05$
3.	20 - 30	19	24	180.3 ± 29.3	177.0 ± 32.6	178.6 ± 31.4	$p > 0.05$
4.	30 - 40	23	13	186.1 ± 28.3	184.2 ± 34.8	185.2 ± 30.6	$p > 0.05$
5.	40 - 50	21	10	178.1 ± 34.8	180.0 ± 33.8	178.7 ± 34.5	$p > 0.05$
6.	50 - 60	16	3	177.8 ± 34.9	197.3 ± 18.6	180.8 ± 35.8	$p > 0.05$
7.	60 - 70	17	4	179.2 ± 40.2	186.8 ± 18.1	180.7 ± 37.8	$p > 0.05$
	Total	129	71	180.9 ± 32.1	180.7 ± 31.9	180.8 ± 32.5	-

*p value is obtained by 't' test.

enzyme level between males and females were found in the age groups of second and sixth decades ($p < 0.05$). In both the age groups males were found to have higher values.

Table 20 depicts the serum ALT level in different age and sex groups. No statistically significant differences were found between male and female of different age groups.

Serum alkaline phosphatase level in different age and sex groups is summarised in Table 21. Statistically significant higher values ($p < 0.05$) were found in the males of second decade age group.

Table 22 shows the serum acid phosphtase level in different age and sex groups. Statistically significant differences between male and female were found in the age group of second and fifth decades ($p < 0.05$). In both age groups males were found to have higher values.

Serum amylase level in different age and sex groups is depicted in Table 23. No statistically significant differences were found between males and females of different age groups.

Important conclusions based on the results discussed above are summarised below:

1. The serum levels of AST, ALT, ALP, ACP and amylase are almost similar in all the four mammalian species.

2. Tissue concentration of these enzymes are much higher than their respective serum levels except in case of amylase.

3. Since in this study consistently similar pattern of tissue distribution of the various enzymes were found in three different mammalian species, namely, buffalo, goat and rabbit, the results can safely be extrapolated to human being.

4. Species dependent differences in the values of any enzyme in a specific tissue varies from 4-50% approximately.

5. The activity of AST is highest in brain followed by heart and skeletal muscle irrespective of species. The enzyme concentration is low in lung.

6. For the same species, ALT level is higher in skeletal muscle, heart and brain and low in liver, lung and serum.

7. ALP concentration is maximum in kidney followed by lung regardless of the species. The levels in heart, brain and skeletal muscle are substantially low.

8. The level of ACP is significantly higher in brain tissue followed by kidney and lung regardless of species.

9. The level of amylase is highest in liver and lowest in skeletal muscle.

10. Amylase activities of lung, brain, heart and serum of any of the species are similar.

11. The normal serum amylase level is significantly on the higher range in Aligarh population.

12. AST and ACP levels in serum are higher in males than in females.

13. The level of ACP in serum is remarkably higher in children than in adult.

14. Serum AST level is higher in males of second and sixth decades than in females of corresponding age group.

15. The levels of ALT and amylase in serum are not dependent on age or sex.

16. The level of ALP in serum is higher in males of second decade than that of the females of corresponding age group.

17. ACP level in the serum is higher in males of second and fifth decades than that of the females of corresponding age group.

SUMMARY

IV. SUMMARY

Tissue enzymes are released in the plasma after cell damage or cell death in a specific organ or tissue. So the elevated level of serum enzymes which are specific for a particular tissue will undoubtedly have a great diagnostic value. The states of health and disease of a specific tissue is also reflected by any changes of the enzymes in the tissue itself. But it is rarely possible to estimate the enzymes in human tissues. However as various metabolic patterns of mammalian species do not differ much from that of human tissues at least qualitatively the data on enzyme levels gathered on tissues of other mammals can be compared to that of human. Furthermore it is an established scientific fact that when consistent results of an experiment are observed in three different mammalian species, the results can safely be extrapolated to human beings. Therefore we have studied the tissue distribution of AST, ALT, ALP, ACP and amylase in buffalo, goat and rabbit. Since the normal levels of serum enzymes are influenced much by the nutritional status, age, sex and ethnic groups (20a), their determination is essential in a country like ours where these values vary substantially from one population group to another. Accordingly we have determined the serum level of the above mentioned enzymes in human.

These enzymes were measured in two hundred blood samples collected from clinically healthy individuals of Aligarh population of different age and sex.

The outcome of this endeavour is described below.

Interestingly there was almost no species dependent differences in the level of AST in serum, skeletal muscle, lung, brain, liver and heart though tissue dependent variations were found. The activity in kidney tissue was nearly the same in goat and buffalo but significantly low in rabbit. The discrepancy can not be attributed to experimental error only. It's significance is not clear to us. The level of AST was found to be highest in brain and low in lung and liver. The activities in heart and skeletal muscle were in between. These findings regarding tissue distribution are in good agreement with the findings (Table 6) of other clinical enzymologists on human tissues except that, the activity found in liver in the present study was much lower than that reported by others (96,103,104). As the method of preparation of enzyme sample was different in all the reported studies such comparison is hardly applicable here.

Species dependent differences in the level of ALT for various tissues range from 4-50%. Maximum difference was noted in lung followed by liver. The enzyme levels were invariant

in the kidney tissue irrespective of species. The serum level of ALT was almost similar in buffalo, goat and human but significantly higher in rabbit. For the same species, the level of ALT was found to be markedly higher in skeletal muscle, heart and brain and substantially lower in liver, lung and serum. Despite experimental inaccuracy these results suggest that ALT level is higher in skeletal muscle, heart and brain.

In heart and serum the ALP levels were almost same regardless of species. Species dependent variation was maximum (52%) in kidney tissue followed by liver, lung, skeletal muscle and brain (25-29%). Except serum the level of enzyme in other tissues was found to be highest in goat followed by buffalo and rabbit. Among the tissues studied here, maximum level was found in kidney. The concentrations in heart, brain and skeletal muscle were significantly low.

The levels of ACP were the same in heart and skeletal muscle for all the three species. Species dependent variation was noted maximum in kidney and intermediate for the other tissues (22-38%). The serum level of the enzyme was found to be very low in all the four species in comparison to other body tissues. Tissuewise highest concentration of the enzyme was noted in brain regardless of species followed by kidney and lung.

The serum amylase levels were found to be similar in all the four mammalian systems. The enzyme levels were substantially higher in liver and kidney irrespective of species. The lowest activity was measured in the skeletal muscle.

The amylase activities of lung, brain, heart and serum were almost similar.

After studying the tissue distribution of these enzymes in different mammalian systems, we measured the normal serum levels of these enzymes in two hundred clinically healthy individuals of Aligarh population.

Statistical analysis of the results shows that the normal serum levels found in this study correlate well with those reported in literature (105-107) except in case of amylase which was found to be significantly on higher normal range in Aligarh population.

Statistically significant sex dependent differences in the enzyme levels were found in case of AST ($p < 0.05$) and ACP ($p < 0.05$) with higher values in male.

The sex dependent differences in the activities of the rest of the enzymes seem to be statistically insignificant ($p > 0.05$). Generally males tend to show somewhat higher level of alkaline phosphatase than women do. This may be related to differences in skeletal mass, but it is also related to

greater physical activity of the male which serves as a stimulus for an increase in bone formation (108,109). Healthy males also exhibit slightly higher transaminase levels than females (33).

Statistically significant higher values of ACP ($p < 0.001$) were found in children (0-12 years) in comparison to adult (12-70 years). Age dependent variations in enzyme levels was insignificant for other enzymes ($p > 0.05$). The level of both acid and alkaline phosphatases is higher in growing children than in adult. This is due to the increased bone growth at this age (109, 110-112). Transaminase level is also higher in children age group (111).

Males in second and sixth decades were found to have statistically significant higher values ($p < 0.05$) of serum AST than that of the females of the same age groups.

Statistically significant higher values of serum ALP ($p < 0.05$) were found in the males of second decade age group.

No statistically significant differences ($p > 0.05$) were fou in serum ALT&amylase levels between males and females of different age groups.

Statistically significant higher values of serum ACP was found in males of second and fifth decade age groups than that of the females of corresponding age groups.

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APPENDIX

APPENDIX - I : SERUM ENZYME PROFILES OF THE STUDY GROUP OF ALIGARH POPULATION.

S.No.	Name	Age yrs.	Sex	AST IU/L	ALT IU/L	ALP KA/dl	ACP KA/dl	Amylase SU/dl
1.	K.	50	F	13	7	5.0	1.2	210
2.	L.S.	20	F	15	6	6.5	1.2	180
3.	R.	14	M	13	16	7.5	1.0	182
4.	M.H.	55	M	20	12	8.0	2.0	210
5.	Z.V.N.	42	M	19	7	5.0	2.7	150
6.	A.H.	35	M	7	9	10.0	1.0	184
7.	S.	16	F	9	7	6.0	1.2	188
8.	S.	25	F	17	6	9.0	1.4	188
9.	S.	40	M	17	6	4.0	1.2	191
10.	H.C.M.	60	M	17	6	4.0	1.0	196
11	K.D.	45	F	20	10	5.0	1.2	180
12.	S.A.	40	M	17	9	11.0	2.0	179
13.	J.P.	40	M	19	14	5.0	1.4	189
14.	N.	60	M	15	10	6.0	1.8	180
15.	M.S.	40	F	13	5	5.5	1.3	188
16.	S.	28	F	15	7	12.0	1.4	176
17.	A	10	M	17	16	11.5	3.0	175
18.	S.B.	62	F	13	7	6.0	1.2	200
19.	A.	4	M	13	10	7.0	2.0	181
20.	B.K.	48	M	15	10	7.5	1.5	176
21.	N.	55	M	13	12	5.5	1.0	185
22.	A.	29	F	15	10	9.0	1.4	150
23.	R.	40	M	7	14	5.0	1.5	215
24.	N.	53	M	11	6	4.0	2.0	170
25.	N	45	F	9	15	8.0	1.0	190
26.	I.F.	27	F	7	12	6.0	1.5	144
27.	M.S.T.	80	M	17	7	7.0	2.0	140
28.	U.	20	F	9	7	5.0	1.0	164
29.	S.	20	F	6	13	7.0	1.6	159
30.	A.M.	60	M	17	7	8.0	2.0	160
31.	K.K.J.	65	M	13	7	5.0	1.7	180
32.	K.	40	M	13	10	5.5	1.0	204
33.	M.	14	F	13	10	5.0	2.0	190
34.	R.T.	27	M	17	7	9.5	1.2	183
35.	S.A.	25	M	19	5	9.0	1.7	174
36.	A.S.	5	M	6	6	6.5	2.2	202
37.	A.N.	15	M	7	7	10.0	1.6	188
38.	A.K.	21	M	7	7	5.0	1.3	190
39.	B.	50	M	9	9	4.5	1.2	183
40.	M.I.	22	M	19	14	10.0	1.3	166

APPENDIX I (Contd.)

S.No.	Name	Age	Sex	AST IU/L	ALT IU/L	ALP KA/dl	ACP KA/dl	Amylase SU/dl
41.	A.B.	15	F	6	7	4.5	1.2	174
42.	H.K.P.	50	M	6	12	11.0	2.2	195
43.	J.P.	75	M	17	7	5.0	4.2	205
44.	P.M.	20	M	11	10	7.5	2.0	196
45.	M.	18	M	20	12	11.0	3.0	193
46.	M	18	F	11	6	9.0	1.3	198
47.	G.	40	M	17	10	5.0	1.2	176
48.	M.I.	65	M	19	6	5.0	1.0	140
49.	M.K.	40	M	15	6	13.0	2.0	189
50.	A.	18	M	13	14	7.0	1.2	205
51.	S.A.H.	25	M	13	12	5.0	1.0	164
52.	M.	10	F	17	9	8.0	1.8	157
53.	M.S.	37	M	9	12	8.0	1.3	168
54.	J.S.	30	M	17	15	5.0	1.0	160
55.	B.S.	53	M	15	10	11.0	1.4	140
56.	R.	40	M	19	12	7.0	1.3	183
57.	B.K.	30	M	11	15	4.0	1.0	198
58.	S.M.	32	M	9	6	9.0	1.2	193
59.	E.A.	20	M	9	7	8.5	1.5	170
60.	B.	21	M	19	7	7.5	1.0	155
61.	N.I.	8	M	19	12	7.0	2.0	166
62.	A.K.	22	M	9	16	7.0	1.2	195
63.	S.	21	F	19	4	5.0	1.2	205
64.	N.B.	30	F	11	7	13.0	1.1	176
65.	Y.K.	10	M	13	7	6.5	2.0	176
66.	A.	35	F	19	15	8.0	1.7	175
67.	G.	16	F	11	14	5.5	1.5	202.
68.	P.	15	F	9	6	5.5	1.2	173
69.	S.	30	F	19	7	10.5	1.2	173
70.	Q	30	M	7	12	13.0	1.5	177
71.	T.	19	M	17	6	7.0	1.8	165
72.	K.S.	35	M	17	12	9.0	1.2	204
73.	V.S.	45	M	15	9	7.0	4.0	201
74.	N.M.	50	M	13	9	8.5	3.5	150
75.	S.A.	24	F	7	14.	8.5	2.0	165
76.	L.A.	17	M	17	14	7.0	1.2	157
77.	H.B.	50	F	15	7	5.0	1.3	160
78.	S.P.	1½	F	10	9	6.0	2.0	185
79.	A.D.	35	F	5	10.	10.0	1.3	193
80.	S.	30	M	15	9	6.0	1.2	196
81.	P.	16	F	7	10	7.0	1.2	164
82.	S.W.	50	F	5	12	10.0	1.0	150
83.	I.D.	24	M	9	6	11.0	1.3	171
84	C.	70	M	17	6	8.5	1.0	172

APPENDIX I (Contd.)

S.No.	Name	Age	Sex	AST IU/L	ALT IU/L	ALP KA/dl	ACP KA/dl	Amylase SU/dl
85.	W.	45	M	17	9	7.0	2.0	175
86.	I.Q.	50	M	19	7	7.0	1.6	183
87.	S.	22	F	13	10	9.0	1.7	170
88.	P.	28	F	15	12	11.0	2.0	195
89.	B.D.	35	F	15	9	10.0	1.6	200
90.	S.S.	40	M	19	10	11.5	1.6	209
91.	D.	8	M	19	8	7.0	2.0	155
92.	AA.K.	20	M	17	16	12.0	1.0	171
93.	H.K.	51	F	10	7	12.0	1.2	210
94.	C.	20	F	19	12	10.0	1.3	175
95.	S.P.	50	M	20	5	10.0	1.5	157
96.	S.	40	F	15	10	8.5	1.2	210
97.	N	80	M	13	9	10.0	1.2	200
98.	L.M.	17	M	17	14	11.0	1.2	200
99.	R.B.	22	F	17	7	10.0	1.4	187
100.	M.L.	60	M	17	7	13.0	1.0	200
101.	A.	35	M	7	9	6.0	12	177
102.	S.S.	70	M	15	10	4.0	1.1	190
103.	S.	40	F	19	14	11.0	1.5	168
104.	B.	55	M	13	10	10.0	1.0	195
105.	S.B.	25	F	18	12	9.0	1.3	183
106.	A.A.	30	M	19	11	9.0	1.5	181
107.	R.	8	F	14	14	11.5	2.0	191
108.	A.A.	42	M	20	7	11.0	1.8	205
109.	K.	30	F	5	6	13.0	1.2	181
110.	Q.	45	F	15	6	6.5	1.3	175
111.	N.S.	61	F	20	10	11.0	2.2	180
112.	R.	30	F	7	9	10.0	1.5	175
113.	R.	30	M	7	7	12.5	1.8	193
114.	S.A.	65	M	19	10	11.5	1.2	160
115.	S.C.S.	40	M	15	6	11.0	2.5	173
116.	M.A.	37	M	19	6	12.0	1.2	166
117.	R.P.S.	48	M	9	15	7.0	1.0	202
118.	B.P.	55	M	20	12	8.0	1.5	180
119.	M.K.	18	M	13	6	7.0	1.3	181
120.	P.K.	24	M	9	6	10.5	1.2	180
121.	R.K.	18	M	11	7	6.5	2.0	177
122.	M.S.	19	M	8	7	10.0	1.2	193
123.	S.	26	F	11	6	9.0	1.0	154
124.	Y.	18	M	13	10	7.0	1.8	190
125.	P.A.	12	M	17	5	11.0	1.5	175
126.	S.A.	65	M	19	10	12.0	1.2	175
127.	S.B.	28	F	15	7	8.0	1.3	180
128.	N.	35	F	17	12	7.5	1.3	157

APPENDIX I (Contd.)

S.No.	Name	Age	Sex	AST IU/L	ALT IU/L	ALP KA/dl	ACP KA/dl	Amylase SU/dl
129.	S.R.	22	F	20	14	10.0	1.2	210
130.	V.K.	14	M	18	9	6.0	1.0	178
131.	J.R.S.	25	M	17	12	9.0	1.4	174
132.	V.K.S.	70	M	18	11	13.0	3.0	185
133.	S.	44	F	11	10	8.0	1.0	180
134.	H.A.H.	68	M	5	5	12.0	2.0	215
135.	R.	18	M	6	12	8.5	1.5	195
136.	M	69	M	14	11	9.0	1.2	180
137.	H	60	M	17	14	11.0	1.1	175
138.	M.L.	32	M	13	9	11.5	1.0	169
139.	G.A.	60	M	13	6	10.0	1.2	160
140.	D.	1½	M	11	9	12.0	2.0	195
141.	S.B.	30	F	19	14	7.0	1.3	195
142.	A.A.	68	M	7	7	11.0	1.3	190
143.	J.B.	40	F	5	6	12.0	2.0	211
144.	S.D.	45	F	19	5	12.0	1.5	185
145.	Z.	25	F	11	9	10.0	1.0	180
146.	H.	45	M	13	9	12.0	1.2	159
147.	K.S.	48	M	9	12	7.0	1.2	181
148.	S.D.	50	F	20	12	12.0	1.3	185
149.	L.K.	15	F	19	10	11.0	1.1	178
150.	R.K.	12	M	19	13.0	8.5	1.3	166
151.	T.	40	F	17	14	12.0	1.4	191
152.	J.S.	50	M	16	9	11.0	1.7	177
153.	H.S.	55	M	19	10	6.0	1.5	155
154.	B.S.	15	M	13	9	8.0	1.3	170
155.	P.K.	35	M	15	7	9.0	1.5	187
156.	V.K.	35	M	19	9	9.5	1.1	177
157.	M.P.	1	M	17	7	12.0	1.6	201
158	N.S.	10	M	15	10	8.0	1.7	183
159	R.	22	M	17	7	7.0	1.4	178
160.	B.B.	70	F	15	6	12.0	1.2	190
161.	P.S.	43	M	17	9	11.0	1.1	163
162.	J.P.	45	M	19	9	9.0	1.3	154
163.	V.K.	19	M	18	13	10.0	1.5	177
164.	M.B.	65	M	15	10	7.0	1.0	170
165.	S.L.	65	M	11	9.	5.0	1.7	160
166.	G.	18	M	19	10	9.0	1.2	181
167.	S.S.	46	M	17	11	13.0	2.5	183
168.	N.J.	45	F	15	10	10.0	1.7	170
169.	S.S.	46	M	17	11	9.0	2.0	189
170.	M.S.	65	M	19	8	10.5	1.0	195

APPENDIX I (Contd.)

S.No.	Name	Age	Sex	AST IU/L	ALT IU/L	ALP KA/dl	ACP KA/dl	Amylase SU/dl
171.	R.B.	55	F	13	6	8.0	1.3	194
172.	K.	18	M	19	8	11.5	1.2	189
173.	M.S.	35	M	18	10	11.0	1.3	188
174.	A.K.	25	F	19	10	11.0	2.0	176
175.	R.L.	33	M	19	6	13.5	1.7	183
176.	R.	38	F	4	6	10.0	1.1	185
177.	N	23	M	9	10	8.0	1.2	150
178.	S.	15	F	11	7	13.0	1.6	184
179.	S.	16	F	6	8	6.5	1.0	190
180.	N	20	M	11	13	5.0	1.4	167
181.	A.K.	30	M	7	7	7.0	1.1	195
182.	L.B.	60	M	17	12	7.5	1.3	172
183.	M.A.	65	M	19	10	8.5	1.5	190
184.	A.Z.	21	F	19	8	7.0	1.1	187
185.	P.S.	60	M	17	9	7.5	2.0	184
186.	B.A.	40	M	13	7	10.0	1.9	205
187.	K.	2	M	9	7	9.5	2.0	188
188.	Z.K.	48	M	13	14	10.0	1.9	201
189.	M.D.	70	F	9	6	12.5	1.0	177
190.	S.B.	30	F	4	11	8.0	1.6	163
191.	M.	30	F	9	6	13.0	1.5	177
192.	S.A.	20	M	7	6	12.0	1.7	179
193.	R.S.	35	F	9	6	5.5	1.5	173
194.	O.B.	25	M	6	7	6.5	1.7	205
195.	M.D.	60	F	7	9	7.0	1.1	188
196.	K.L.	45	M	6	9	7.0	1.6	178
197.	G.S.	60	M	7	6	7.5	1.2	188
198.	B.	35	F	11	9	5.0	1.1	154
199.	S.A.	40	M	9	7	10.0	1.6	205
200.	N.	25	F	17	14	8.0	1.2	194